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<b>(54) Title:</b> HUMAN BREAST AND OVARIAN CANCER ASSOCIATED GENE SEQUENCES AND POLYPEPTIDES		
<b>(57) Abstract</b>  <p>This invention relates to newly identified breast, ovarian, breast cancer and/or ovarian cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "breast/ovarian cancer antigens", and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such breast/ovarian cancer antigens for detection, prevention and treatment of disorders of the female reproductive system, particularly disorders of the breast and/or ovary, including the presence of breast cancer and/or ovarian cancer. This invention relates to the breast/ovarian cancer antigens as well as vectors, host cells, antibodies directed to breast/ovarian cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the female reproductive system, particularly disorders of the breast and/or ovary, including breast cancer and/or ovarian cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of breast/ovarian cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.</p>		

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## **Human Breast and Ovarian Cancer Associated Gene Sequences and Polypeptides**

### **5    *Field of the Invention***

This invention relates to newly identified breast, ovarian, breast cancer, and ovarian cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "breast/ovarian cancer antigens," and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such  
10    breast/ovarian cancer antigens for detection, prevention and treatment of disorders of the female reproductive system, specifically disorders of the breast or ovary, particularly the presence of breast and/or ovarian cancer. This invention relates to the breast/ovarian cancer antigens as well as vectors, host cells, antibodies directed to breast/ovarian cancer antigens and recombinant and synthetic methods for producing the same. Also provided are  
15    diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the female reproductive system, specifically disorders of the breast and/or ovary, including breast cancer and/or ovarian cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of breast/ovarian cancer antigens of the invention. The present invention further  
20    relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

### ***Background of the Invention***

Breast cancer represents the most frequent cause of early morbidity and mortality in  
25    women in North America (Harris et al, New Eng. J. Med. 327:319, 390 and 473 (1992)). It is generally believed that this malignancy arises from a multi step process involving mutations in a relatively small number of genes, perhaps 10 or less. These mutations result in significant changes in the growth and differentiation of breast tissue that allow it to grow independent of normal cellular controls, to metastasize, and to escape immune surveillance. The genetic  
30    heterogeneity of most breast cancers suggests that they arise by a variety of initiating events

and that the characteristics of individual cancers are due to the collective pattern of genetic changes that accumulate (Harris et al. *New Eng. J. Med.* 327:319, 390 and 473 (1992)).

The classes of genes that are involved in breast cancer are not unlike those found in a number of other well characterized malignancies, although some are highly specific for breast cancer. In particular, mutations in the genes that encode receptors involved in binding to estrogen and progesterone are particularly important because they likely cause the breast cells to proliferate while rendering them unresponsive to the antitumor effects of these hormones in advanced malignancy. In addition, changes in the genes that encode growth factors, other receptors, signal transduction molecules, and transcription factor molecules are frequently involved and have alterations that are involved in the development and progression of breast cancer (King, *Nature Genetics* 2:125 (1992)). The characterization of the type and number of mutations seen in individual breast cancers is useful in classifying the biological properties of individual cancers and in determining the prognosis for individual patients. For example, the *erbB2/HER2/neu* gene is particularly valuable in predicting the prognosis of both node-positive and node-negative patients based on the amplification status of the gene (King, *Science* 250:1684 (1990)). Several additional members of this family have been discovered but the ligand for *erbB2/HER2/neu* remains unknown. It is anticipated that further advances in therapeutics will be achieved by the development of therapies that disrupt aberrant growth signaling pathways or affect the cellular interactions of breast cancer cells with native stroma or metastatic sites.

Although oncogenes are likely to be very important in breast cancer, tumor suppressor genes may also play an important role. Certain of these genes, including *p53* and *Rb-1*, are essential to the normal mechanisms that control cell cycle events, especially those checkpoints at the border of the different stages of the cell cycle (Hollstein et al, *Science* 253:49 (1991); Srivastava et al, *Nature* 348:747 (1990)).

In 1969, Li and Fraumeni documented a familial cancer syndrome that had an autosomal dominant pattern of expression (Li et al, *Ann. Intern. Med.* 71:747 (1969)). Members of these families had sarcomas, breast cancers, brain tumors, leukemias, adrenocortical carcinomas, and other malignancies. Family studies demonstrated that the gene responsible for the syndrome was located on chromosome 17, and examination of the *p53* gene as a candidate gene revealed that this gene was mutated in five families (Malsin et al, *Science* 250:1233 (1990)). In the last two years, two genes linked to familial breast cancer,



designated BRCA1 and BRCA2, have been isolated and characterized. BRCA1 is at 17q21 (Claus et al, Am. J. Epidemiology 131:961 (1990); Hall et al, Science 250:1684 (1990); Easton et al, Am. J. of Human Genetics 52 (4):678 (1993); Black et al, Am. J. of Human Genetics 52 (4):702 (1993); Bowcock et al, Am. J. of Human Genetics 52 (4):718 (1993); Miki et al, Science 266:66 (1995)). The demonstration of loss of heterozygosity (LOH) at 17q25 has defined another potential tumor suppressor gene (Lindblom et al, Human Genetics 91:6 (1993); Cornelis et al, Oncogene 8:781 (1993); Theile et al, Oncogene 10:439 (1995)).

There is a need, therefore, for identification and characterization of such factors that modulate activation and differentiation of breast and ovarian cells, both normally and in disease states. In particular, there is a need to isolate and characterize additional molecules that mediate apoptosis, DNA repair, tumor-mediated angiogenesis, genetic imprinting, immune responses to tumors and tumor antigens and, among other things, that can play a role in detecting, preventing, ameliorating or correcting dysfunctions or diseases.

The present invention relates at least in part, to a novel breast and ovarian and breast and ovarian cancer related polynucleotides and polypeptides. The discovery of these breast and ovarian cancer related polynucleotides provides new compositions which are useful in the diagnosis, prevention and treatment of disorders of the female reproductive system, particularly of the ovary including, but not limited to ovarian cancer, and the breast, including but not limited to breast cancer.

### ***Summary of the Invention***

The present invention includes isolated nucleic acid molecules comprising, or alternatively, consisting of, a breast, ovarian, breast cancer and/or ovarian cancer associated polynucleotide sequence disclosed in the sequence listing (as SEQ ID Nos:1 to 418) and/or contained in a human cDNA clone described in Tables 1, 2 and 5 and deposited with the American Type Culture Collection ("ATCC"). Fragments, variant, and derivatives of these nucleic acid molecules are also encompassed by the invention. The present invention also includes isolated nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide encoding a breast, ovarian, breast cancer, and/or ovarian cancer polypeptide. The present invention further includes breast, ovarian, breast cancer, and/or ovarian cancer polypeptides encoded by these polynucleotides. Further provided for are amino acid

sequences comprising, or alternatively consisting of, breast, ovarian, breast cancer, and/or ovarian cancer polypeptides as disclosed in the sequence listing (as SEQ ID Nos: 419 to 836) and/or encoded by a human cDNA clone described in Tables 1, 2 and 5 and deposited with the ATCC. Antibodies that bind these polypeptides are also encompassed by the invention.

5 Polypeptide fragments, variants, and derivatives of these amino acid sequences are also encompassed by the invention, as are polynucleotides encoding these polypeptides and antibodies that bind these polypeptides. Also provided are diagnostic methods for diagnosing and treating, preventing, and/or prognosing disorders related to the female reproductive system, specifically disorders related to the breast and/or ovary, including breast cancer

10 and/or ovarian cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of breast/ovarian cancer antigens of the invention.

### *Detailed Description*

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#### **Tables**

Table 1 summarizes some of the breast/ovarian cancer antigens encompassed by the invention (including contig sequences (SEQ ID NO:X) and the cDNA clone related to the contig sequence) and further summarizes certain characteristics of the breast/ovarian cancer polynucleotides and the polypeptides encoded thereby. The first column shows the "SEQ ID NO:" for each of the 418 breast/ovarian cancer antigen polynucleotide sequences of the invention. The second column provides a unique "Sequence/Contig ID" identification for each breast, ovarian, breast cancer and/or ovarian cancer associated sequence. The third column, "Gene Name," and the fourth column, "Overlap," provide a putative identification

20 of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database and the database accession no. for the database sequence having similarity, respectively. The fifth and sixth columns provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as

25 SEQ ID NO:Y. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity), respectively, observed between the aligned sequence

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segments of the translation product of SEQ ID NO:X and the database sequence. The ninth column provides a unique "Clone ID" for a cDNA clone related to each contig sequence.

Table 2 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application.

5 Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, fifteen or more of any one or more of these public EST sequences are optionally excluded from certain embodiments of the invention.

10 Table 4 lists residues comprising antigenic epitopes of antigenic epitope-bearing fragments present in most of the breast, ovarian, breast cancer or ovarian cancer associated polynucleotides described in Table 1 as predicted by the inventors using the algorithm of Jameson and Wolf, (1988) Comp. Appl. Biosci. 4:181-186. The Jameson-Wolf antigenic analysis was performed using the computer program PROTEAN (Version 3.11 for the Power MacIntosh, DNASTAR, Inc., 1228 South Park Street Madison, WI). Breast, ovarian, breast cancer and/or ovarian cancer associated polypeptides (e.g., SEQ ID NO:Y, polypeptides  
15 encoded by SEQ ID NO:X, or polypeptides encoded by the cDNA in the referenced cDNA clone) may possess one or more antigenic epitopes comprising residues described in Table 4. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly. The residues and locations shown in column two of Table 4 correspond to the amino acid sequences for most  
20 breast, ovarian, breast cancer and/or ovarian cancer associated polypeptide sequence shown in the Sequence Listing.

Table 5 shows the cDNA libraries sequenced, and ATCC designation numbers and vector information relating to these cDNA libraries.

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### **Definitions**

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

30 In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be

"isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X (as described in column 1 of Table 1) or the related cDNA clone (as described in column 9 of Table 1 and contained within a library deposited with the ATCC). For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

In the present invention, "SEQ ID NO:X" was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X is deposited at Human Genome Sciences, Inc. (HGS) in a catalogued and archived library. As shown in column 9 of Table 1, each clone is identified by a cDNA Clone ID. Each Clone ID is unique to an individual clone and the Clone ID is all the information needed to retrieve a given clone from the HGS library. In addition to the individual cDNA clone deposits, most of the cDNA libraries from which the clones were derived were deposited at the American Type Culture Collection (hereinafter "ATCC"). Table 5 provides a list of the deposited cDNA libraries. One can use the Clone ID to determine the library source by reference to Tables 2 and 5. Table 5 lists the deposited cDNA libraries by name and links each library to an ATCC Deposit. Library names contain four characters, for example, "HTWE." The name of a cDNA clone ("Clone ID") isolated from that library begins with the same four characters, for example "HTWEP07". As mentioned below, Table 1 correlates the Clone ID names with SEQ ID NOs. Thus, starting with a SEQ ID NO, one can use Tables 1, 2 and 5 to determine the corresponding Clone ID, from which library it came and in which ATCC deposit the library is contained. Furthermore,

it is possible to retrieve a given cDNA clone from the source library by techniques known in the art and described elsewhere herein. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein), and/or sequences contained in the related cDNA clone within a library deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also included within "polynucleotides" of the present invention are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA<sup>+</sup> sequences (such as any 3' terminal polyA<sup>+</sup> tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

"SEQ ID NO:X" refers to a breast/ovarian cancer antigen polynucleotide sequence described in Table 1. SEQ ID NO:X is identified by an integer specified in column 1 of Table 1. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF)

encoded by polynucleotide SEQ ID NO:X. There are 418 breast/ovarian cancer antigen polynucleotide sequences described in Table 1 and shown in the sequence listing (SEQ ID NO:1 through SEQ ID NO:418). Likewise there are 418 polypeptide sequences shown in the sequence listing, one polypeptide sequence for each of the polynucleotide sequences (SEQ ID NO:419 through SEQ ID NO:836). The polynucleotide sequences are shown in the sequence listing immediately followed by all of the polypeptide sequences. Thus, a polypeptide sequence corresponding to polynucleotide sequence SEQ ID NO:1 is the first polypeptide sequence shown in the sequence listing. The second polypeptide sequence corresponds to the polynucleotide sequence shown as SEQ ID NO:2, and so on. In other words, since there are 418 polynucleotide sequences, for any polynucleotide sequence SEQ ID NO:X, a corresponding polypeptide SEQ ID NO:Y can be determined by the formula  $X + 418 = Y$ . In addition, any of the unique "Sequence/Contig ID" defined in column 2 of Table 1, can be linked to the corresponding polypeptide SEQ ID NO:Y by reference to Table 4.

The polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation,

hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

The breast, ovarian, breast cancer and/or ovarian cancer polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The breast, ovarian, breast cancer and/or ovarian cancer polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to



a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

"A polypeptide having functional activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The functional activity of the breast/ovarian cancer antigen polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to an antibody to the full length polypeptide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See

generally, Phizicky, E., et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants derivatives and analogs thereof to elicit polypeptide related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

#### **Breast, Ovarian, Breast Cancer and Ovarian Cancer Associated Polynucleotides and Polypeptides of the Invention**

It has been discovered herein that the polynucleotides described in Table 1 are expressed at significantly enhanced levels in human breast, ovarian, breast cancer and/or ovarian cancer tissues. Accordingly, such polynucleotides, polypeptides encoded by such polynucleotides, and antibodies specific for such polypeptides find use in the prediction, diagnosis, prevention and treatment of disorders related to the female reproductive system, specifically disorders of the breast and/or ovary, including breast cancer and/or ovarian cancer as more fully described below.

Table 1 summarizes some of the polynucleotides encompassed by the invention (including contig sequences (SEQ ID NO:X) and the related cDNA clones) and further summarizes certain characteristics of these breast, ovarian, breast cancer and/or ovarian cancer associated polynucleotides and the polypeptides encoded thereby.

Table 1

Seq ID No.	Sequence/ Contig ID	Gene Name	Overlap	HGS Nucleotide		% Identity	% Similarity	Clone ID
				Start	End			
1	419266	monoamine oxidase B [Homo sapiens] >gi 187376 monoamine oxidase B [Homo sapiens] >bbs 13402  monoamine oxidase B, MAO B [human, platelet, Peptide Partial, 520 aa] [Homo sapiens] >pir JH0817 H0817 amine oxidase (flavin-containing) (EC 1.4.3.4) B - human >	gi 187359	2	1021	95	95	HAGFP75
2	429114			51	383			HATDC43
3	506777			51	233			HRGCY74
4	508678	(AF059293) cytokine-like factor-1 precursor [Homo sapiens] >sp O75462 O75462 CYTOKINE-LIKE FACTOR-1 PRECURSOR. Length = 422	gi 3372627	3	155	100	100	HFJUG81
5	508968	DNA helicase [Homo sapiens] >pir A58836 A58311 DNA helicase RECQL - human Length = 659	gi 619863	2	739	95	96	HHTLH91
6	509029			770	1096			HLMDG72
7	519726			359	529			HCSB83

8	522632		3	299		HRGBG45
9	524655		522	686		HUSGS36
10	525847	glyoxalase II [Homo sapiens] >sp Q16775 GLO2_HUMAN HYDROXYACYLGLUTATHIONE HYDROLASE (EC 3.1.2.6) (GLYOXALASE II) (GLX II). Length = 260	gnl PID e1971 27	1	162	54 73 H6EDP14
11	530306		239	355		HCHCC28
12	532818	(AF035178) elongation factor 1 A2 [Oryctolagus cuniculus] >gi 38456 elongation factor 1 alpha-2 [Homo sapiens] >pir S35033 EFHUA2 translation elongation factor eEF-1 alpha-2 chain - human >sp Q05639 EF12_HUMAN ELONGATION FACTOR 1-ALPHA 2 (EF-1-ALPHA-2) (S	gi 3098311	43	441	95 HAMFD92
13	533385		1258	1827		HTWAO42
14	533532	actin capping protein alpha subunit [Homo sapiens] >gi 2393732 (AC002543) f-actin capping protein alpha-2 subunit [Homo sapiens] >sp P47755 CAZ2_HUMAN F- ACTIN CAPPING PROTEIN ALPHA-2 SUBUNIT (CAPZ). >gi 433308 capping protein alpha [Homo sapiens] {SUB 3-2	gi 595255	18	947	95 HETCD42

15	534852	(AF041472) ataxin-2 [Mus musculus] >sp O70305 O70305 SPINOCEREBELLAR ATAXIA 2 HOMOLOG (ATAXIN-2). Length = 1285 R kappa B [Homo sapiens] >pir S52863 S52863 DNA-binding protein R kappa B - human >sp Q15312 Q15312 R KAPPA B. Length = 1324	gil3005020	3	869	77	77	HCE4Q55
16	537910		gil695579	3	443	100	100	HTOAO52
17	538460			574	1026			HSSMY42
18	539577	transcriptional activator [Homo sapiens] >gnl PID d1005685 hSNF2b [Homo sapiens] >pir S45252 S45252 SNF2beta protein - human >gil4056413 (AC006127) SN24_HUMAN; nuclear protein GRB1; homeotic gene regulator; SNF2-BETA [Homo sapiens] {SUB 814-1474} Length =	gil902046	1	540	89	89	HKADQ93
19	548379	complement protein C7 precursor [Homo sapiens] >pir A27340 A27340 complement C7 precursor - human >sp P10643 CO7_HUMAN COMPLEMENT COMPONENT C7 PRECURSOR. Length = 843 proteasome subunit HsN3 [Homo sapiens] >pir S50147 S50147 multicatalytic endopeptidase complex (EC 3.4.99.46) beta chain N3 - human >sp P28070 PRCB_HUMAN PROTEASOME BETA CHAIN PRECURSOR (EC 3.4.99.46) (MACROPAIN BETA CHAIN)	gil179716	92	1336	92	92	HAATCK25
20	548489		gnl PID d100 6192	3	857	99	99	HCGAF33

(MULTICATALYTIC ENDOPEPTIDASE  
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21	548595	inosine monophosphate dehydrogenase type II [Homo sapiens] >gi 1702964 inosine monophosphate dehydrogenase type II [Homo sapiens] >pir 52303 A31997 IMP dehydrogenase (EC 1.1.1.205) II - human >sp P12268 IMD2_HUMAN INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE	gi 602458	971	1525	100	100	HIXEE92
22	549337	stromelysin-3 precursor [Homo sapiens] Length = 488	gi 456257	449	1081	96	96	IJMAF23
23	549777			54	293			HPMAC61
24	553091	pancreatic peptidylglycine alpha-amidating monooxygenase, PAM=membrane-bound isoform {alternatively spliced, clone PAM-3, transmembrane domain (Ba region)} [human, islet cell tumor cell line QGP-1, Peptide Partial, 971 aa] [Homo sapiens] >sp Q16252 Q16252	bbs 159681	898	2598	97	97	HEMFU73
25	553827	B-CAM gene product [Homo sapiens] >pir 37202 37202 B-CAM protein - human Length = 588	gi 535179	2	388	80	80	HBHMI67

26	556350		263	655			HCHOC59	
27	556351	'FKBP52; 52 kD FK506 binding protein' [Homo sapiens] >pir A46372 A46372 immunophilin FKBP52 - human >sp Q02790 FKB4_HUMAN P59 PROTEIN (HSP BINDING IMMUNOPHILIN) (HBI) (POSSIBLE PEPTIDYL-PROLYL CIS-TRANS ISOMERASE) (EC 5.2.1.8) (PPIASE) (ROTAMASE) (FKBP5 ubiquitin conjugating enzyme [Homo sapiens] >pir A49630 A49630 ubiquitin conjugating enzyme - human (fragment) Length = 298 (AD001530) putative [Homo sapiens] >sp G2335055 G2335055 XAP-5. >gn PID1012538 HXC-26 [Homo sapiens] {SUB 15-339} >gi 1203974 XAP-5 gene product [Homo sapiens] {SUB 66-339} Length = 339 adipocyte lipid-binding protein [Homo sapiens] >pir A33363 FZHUF fatty acid-binding protein, adipocyte - human >sp P15090 FABA_HUMAN FATTY ACID-BINDING PROTEIN, ADIPOCYTE (AFABP) (ADIPOCYTE LIPID-BINDING PROTEIN) (ALBP) (A-FABP). {SUB 2-132} Length = 132 N-cadherin [Homo sapiens] Length = 747	gi 186390	2	1216	97	97	HE8DF57
28	557007		gi 388309	3	698	99	100	HTEJK85
29	558140		gi 2335055	3	1070	71	71	HKAAM18
30	558456		gi 178347	69	332	100	100	HISBQ67
31	558708		gi 416293	3	515	79	79	HSYBX61
32	574789			301	402			HLDNM79

33	578203			2	445				H6EDN57
34	585385	precursor polypeptide (AA -21 to 782) [Homo sapiens] >pir A35954 A35954 endoplasmic precursor - human >sp P14625 ENPL_HUMAN ENDOPLASMIN PRECURSOR (94 KD GLUCOSE-REGULATED PROTEIN) (GRP94) (GP96 HOMOLOG) (TUMOR REJECTION ANTIGEN 1). Length = 803 leukocyte adhesion glycoprotein precursor [Homo sapiens] Length = 1152	gi 37261	99	347	71	71		1101MP70
35	588869		gi 307114	1	720	98	98		HDPEK39
36	597076	preferentially expressed antigen of melanoma [Homo sapiens] >sp P78395 P78395 PREFERENTIALLY EXPRESSED ANTIGEN OF MELANOMA. Length = 509 sigma receptor [Homo sapiens] >gi 1916800 SR31747 binding protein 1 [Homo sapiens] >gi 2914740 (AF001977) type I sigma receptor [Homo sapiens] >pir JC5266 JC5266 sigma receptor 1 - human >sp Q99720 Q99720 SIGMA RECEPTOR. Length = 223	gi 1903384	80	811	77	77		H1ETH166
37	598656		gi 1783387	3	587	100	100		HMEIY05



38	611880	Acetyl-CoA:acetyltransferase (EC 2.3.1.9) (Acetoacetyl-CoA thiolase). [Escherichia coli] >gil1788554 (AE000311) acetyl-CoA acetyltransferase [Escherichia coli] >pir1F649921F64992 hypothetical protein b2224 - Escherichia coli (strain K-12) >sp1P76461 ATOB_	gnl PID d1016745	1	108	100	100	HOVAS88
39	614329	ORF, HEIR-1; pot. neuroblastoma-associated regulator [Homo sapiens] >gil395338 helix-loop-helix protein [Homo sapiens] >gil512437 HEIR-1 [Homo sapiens] {SUB 30-148} Length = 148	gil490013	300	755	86	86	HIFPCQ02
40	616066			121	213			HSIGC05
41	620956	ribosomal protein S9 [Rattus norvegicus] >pirJN0587 S21497 ribosomal protein S9 - rat Length = 194	gil57143	3	473	95	97	HOF0B28
42	621889	unnamed protein product [unidentified] >gil468550 CCT (chaperonin containing TCP-1) epsilon subunit [Mus musculus] >pir S43061 S43061 t-complex-type molecular chaperone Ccte - mouse Length = 541	gnl PID e306129	16	423	95	97	HOF0C44
43	624017	(AB003732) polyubiquitin [Cricetulus griseus] >sp O35080 O35080 POLYUBIQUITIN. >gil4105408 (AF045474) polyubiquitin [Schistosoma mansoni] {SUB 694-988} Length = 1038	gil2627133	1	1170	95	97	HMCBS12

44	651784	histone H2A.X [Homo sapiens] >pir S07631 S07631 histone H2A.X - human >sp P16104 H2AX_HUMAN HISTONE H2A.X. {SUB 2-143} Length = 143	gi 31973	2	514	98	98	HKGA194
45	651826	keratin, 55K type II cytoskeletal - human (fragment) Length = 489	pir B24177 B 24177	2	1300	86	86	HNTAH42
46	653282	phosphate transfer protein B precursor, mitochondrial - bovine Length = 361	pir D53737 D 53737	30	392	90	90	HOFNY90
47	657122			1	204			HKGAQ13
48	661442	rab1B protein (AA 1 - 201) [Rattus sp.] Length = 201	gi 57006	1	672	98	99	IICHM133
49	664914	phosphotyrosyl phosphatase activator [Oryctolagus cuniculus] >pir B54021 B54021 phosphotyrosyl phosphatase activator PTPA - rabbit >sp Q28717 Q28717 PHOSPHOTYROSYL PHOSPHATASE ACTIVATOR. Length = 323	gi 509144	1	228	98	100	HEGAK11
50	666654			63	395			HOFNL37
51	667084	cytokeratin 17 [Homo sapiens] >gi 34075 keratin related product [Homo sapiens] >pir S30433 S30433 keratin 17, cytoskeletal - human >sp Q04695 K1CQ_HUMAN KERATIN, TYPE I CYTOSKELETAL 17 (CYTOKERATIN 17) (K17) (CK 17) (39.1) (VERSION 1). {SUB 2-432} Length	gi 30379	3	1379	100	100	HKADA74

52	667380	cell surface glycoprotein [Homo sapiens] >gnl PID d1006754 TALLA-1 [Homo sapiens] >gnl PID d1001976 cell surface glycoprotein [Homo sapiens] >pir 139368 139368 T-cell acute lymphoblastic leukemia associated antigen 1 - human >sp P41732 A15_HUMAN CELL SURF	gnl PID d1001976	1	474	100	100	HMIBK53
53	669530			264	440			11PFCJ30
54	671315	cell cycle checkpoint control protein [Homo sapiens] >sp Q99638 Q99638 CELL CYCLE CHECKPOINT CONTROL PROTEIN. Length = 391	gi 1765956	320	1279	92	92	HDABE95
55	671993	NAD(H)-specific isocitrate dehydrogenase gamma-subunit precursor [Homo sapiens] >gnl PID e219959 NAD (H)-specific isocitrate dehydrogenase gamma subunit precursor [Homo sapiens] >gi 1302655 NAD+-isocitrate dehydrogenase gamma subunit [Homo sapiens] >gi 40	gnl PID e211919	1	993	91	91	11SICA89
56	674618			223	312			HOVBX22
57	675027			789	1160			HSDII69
58	677202	vimentin [Homo sapiens] >sp Q15867 Q15867 VIMENTIN (FRAGMENT). Length = 354	gi 340232	705	896	100	100	HWACG51

59	678504	ORF YGR031w [Saccharomyces cerevisiae] >pir S64322 S64322 probable membrane protein YGR031w - yeast (Saccharomyces cerevisiae) Length = 342	gnl PID e243277	320	640	38	63	HCHAG27
60	678985	54 kDa protein [Homo sapiens] >gnl PID e1245514 p54nrb [Homo sapiens] >pir G01211 G01211 54 kDa protein - human >sp Q12786 Q12786 54 KDA PROTEIN. Length = 471	gij407308	358	1203	100	100	HCI10L54
61	682161	(AF036241) Na+/H+ exchange regulatory co-factor [Homo sapiens] >gij3220019 (AF015926) ezrin-radixin-moesin binding phosphoprotein-50 [Homo sapiens] >sp O14745 O14745 EZRIN-RADIXIN-MOESIN BINDING PHOSPHOPROTEIN-50. Length = 358	gij2920585	3	869	89	89	HCHAG19
62	683476			1	132			II0FMM27
63	691146	KDEL receptor [Homo sapiens] >pir S13293 S13293 KDEL receptor - human >sp P24390 ER21_HUMAN ER LUMEN PROTEIN RETAINING RECEPTOR 1 (KDEL RECEPTOR 1). Length = 212	gij34031	1	372	100	100	IIDA1B102
64	693589			1	393			HCHAS12

65	694991	B4B gene product [Homo sapiens] >gnl PID e1949 protein [Homo sapiens] >gil1932786 epithelial membrane protein [Homo sapiens] >gil2506160 TMP [Homo sapiens] >sp P54849 EMPI_HUMAN EPITHELIAL MEMBRANE PROTEIN-1 (EMP-1) (TUMOR-ASSOCIA	gnl PID e1949 46	1	663	98	98	HRAAY77
66	698303	heat shock factor 1 [Homo sapiens] >pir A41137 A41137 heat shock transcription factor 1 - human >sp Q00613 HSF1_HUMAN HEAT SHOCK FACTOR PROTEIN 1 (HSF 1) (HEAT SHOCK TRANSCRIPTION FACTOR 1) (HSTF 1). Length = 529 filamin [Homo sapiens] Length = 2647	gil184403	23	1168	85	85	HSHCA55
67	698669		gil1203969	27	1274	98	98	HEGAR20
68	705696			321	458			HOFMP28
69	706393	vacuolar H+ ATPase proton channel subunit [Homo sapiens] >pir A39367 A39367 H+- transporting ATPase (EC 3.6.1.35) chain PKD1 - human Length = 155	gil189676	119	604	84	85	HSKHP64
70	707357			3	344			HOFMM35

71	707360	leucine aminopeptidase, LAP [cattle, kidney, Peptide, 513 aa] [Bos taurus] >pir A54338 APBOL_Leucyl aminopeptidase (EC 3.4.11.1), renal - bovine >sp P00727 AMPL_BOVIN CYTOSOL AMINOPEPTIDASE (EC 3.4.11.1) (LEUCINE AMINOPEPTIDASE) (LAP) (LEUCYL AMINOPEPTIDA	bbs 137417	1	447	81	89	HOFOF35
72	707375	serine/threonine protein kinase [Homo sapiens] >pir S23385 S23385 protein kinase (EC 2.7.1.37) cdc2-related PCTAIRE-1 - human >sp Q00536 KPT1_HUMAN SERINE/THREONINE-PROTEIN KINASE PCTAIRE-1 (EC 2.7.1.-). >sp G252370 G252370 CDC2-RELATED PROTEIN KINASE {CL	gi 36619	2	1582	92	92	HTOJQ73
73	707754			2	376			HLDBT45
74	711172			237	395			HOVC140
75	712248	transcription factor AP-2 beta [Homo sapiens] >sp E286536 E286536 TRANSCRIPTION FACTOR AP-2 BETA. Length = 367	gn PID e286536	99	344	100	100	HKGCW94
76	715445	DNA-PK [Homo sapiens] >pir G02083 G02083 DNA-PK - human (fragment) >sp Q13337 Q13337 DNA-PK (FRAGMENT). Length = 930	gi 1017757	119	988	99	99	HLTDJ07
77	716362			221	688			HGBGC77

78	716835	(AF036241) Na+/H+ exchange regulatory co-factor [Homo sapiens] >gi 3220019 (AF015926) ezrin-radixin-moesin binding phosphoprotein-50 [Homo sapiens] >sp O14745 O14745 EZRIN-RADIXIN-MOESIN BINDING PHOSPHOPROTEIN-50. Length = 358	gi 2920585	3	755	79	79	HCHAI81
79	716947	SRp55-2 [Homo sapiens]. Length = 135	gi 1049084	2	145	100	100	HADDY71
80	717685	alpha-mannosidase [Homo sapiens] Length = 987	gi 1419374	2	1120	99	99	IIDPUO15
81	719755			89	802			HCGACS4
82	720389	inducible membrane protein [Homo sapiens] >gi 806806 cell surface glycoprotein [Homo sapiens] >gi 1832296 metastasis suppressor [Homo sapiens] >pir 138942 A46493 metastasis suppressor KAI1 - human >sp P27701 CD82_HUMAN CD82 ANTIGEN (INDUCIBLE MEMBRANE PRO	gi 35833	1	594	65	67	HUVCR41
83	720903	cDNA isolated for this protein using a monoclonal antibody directed against the p27k prosomal protein [Homo sapiens] Length = 266	gnl PID e103161	108	614	93	95	HFV1H35

84	721348	G6PD (AA 1-515) [Homo sapiens] >sp P11413 G6PD_HUMAN GLUCOSE-6-PHOSPHATE 1-DEHYDROGENASE (EC 1.1.1.49) (G6PD). {SUB 2-515} >gil439445 glucose-6-phosphate dehydrogenase [Didelphis virginiana] {SUB 258-288} >sp O46666 O46666 GLUCOSE-6-PHOSPHATE DEHYDROGENAS	gil31543	545	2065	93	93	HSIBL14
85	721562	pescadillo [Homo sapiens] >sp O00541 O00541 PESCADILLO. Length = 588	gil2194203	32	811	99	99	HCFCCK84
86	722775			409	1680			HCIHAD52
87	724463			126	335			HOFMP50
88	727501	SWI/SNF complex 170 KDa subunit [Homo sapiens] >sp Q92923 Q92923 SWI/SNF COMPLEX 170 KDA SUBUNIT. Length = 1213	gil1549241	1	1302	97	97	HL YBV46
89	728418	GTP binding protein [Mus musculus] >pir A39611 A39611 probable GTP-binding protein - mouse >sp P23249 MV10_MOUSE PROTEIN MOV-10. >gil433685 gb 110/Mov 10 locus gene product [Mus musculus] {SUB 1-45} Length = 1004	gil53169	3	911	93	96	HSSEP09
90	728920	adipophilin [Homo sapiens] >sp Q99541 Q99541 ADIPOPHILIN (FRAGMENT). Length = 437	gnl PID e292752	2	751	89	89	HLDRQ71
91	732958			3	296			HP1YA52



92	733134	NF45 protein [Homo sapiens] >pir A54857 A54857 transcription factor NF-AT 45K chain - human >sp Q12905 Q12905 NF45 PROTEIN. Length = 406	gi 532313	84	1259	100	100	HHBHP80
93	734099			150	365			HBGDI44
94	734599			163	705			H6EED05
95	736019	ribosomal protein L11 [Homo sapiens] >gi 57678 ribosomal protein L11 [Rattus rattus] >pir S17351 R5RT11 ribosomal protein L11 precursor - rat >sp G3115334 G3115334 RIBOSOMAL PROTEIN L11. >sp D1026769 D1026769 RIBOSOMAL PROTEIN L11 (FRAGMENT). {SUB 17-52}	gi 3115334	3	608	100	100	HSEBB02
96	738268			45	233			HE20C41
97	738911	(AF069291) hT41 [Homo sapiens] >sp G3687829 G3687829 HT41. Length = 505	gi 3687829	3	656	40	62	HCHCI12
98	739226			3	125			HADFY59
99	739527			3	752			HAACCL62
100	740710	acyl-CoA synthetase-like protein [Homo sapiens] Length = 670	gnl PID c3212 96	8	307	96	100	HPMFQ72

101	742980	serine-threonine specific protein phosphatase [Homo sapiens] >sp E1334695 E1334695 SERINE-THREONINE SPECIFIC PROTEIN PHOSPHATASE (EC 3.1.3.16). Length = 317	gnl PID e1334695	3	182	81	86	HSKCE51
102	744331	ZINC FINGER PROTEIN {N-TERMINAL}. Length = 77	sp G632682 G632682	432	791	62	80	HCHAH75
103	744751	collagen alpha 3(VI) chain precursor - human Length = 2970	pir S13679 C GHU3A	902	1189	100	100	IUFV63
104	745750			349	714			ICEHX66
105	746285			2016	2297			HINTNQ78
106	746416	(AB013357) 49 kDa zinc finger protein [Mus musculus] Length = 460	gnl PID d1038083	113	391	97	97	HOFMO90
107	747851	(AF035387) C7-1 protein [Rattus norvegicus] >sp O54715 O54715 C7-1 PROTEIN. Length = 463	gi 2655418	3	974	78	80	HSSJG21
108	750632			252	449			HOGBF68
109	751315			423	608			HLTGN10
110	754009			408	773			HE8PN81
111	754634			525	1070			HUSGI70
112	756637	(AF044127) peroxisomal short-chain alcohol dehydrogenase [Homo sapiens] >sp G4105190 G4105190 PEROXISOMAL SHORT-CHAIN ALCOHOL DEHYDROGENASE. Length = 260	gi 4105190	38	586	89	91	HMWY27

113	756833		1	387		HCEDP17
114	756878		127	399		HHBDE92
115	757332	cytokeratin 8 [Homo sapiens] >gi 553163 keratin 8 [Homo sapiens] {SUB 1-231} Length = 482	35	235	96	HOFMI52
116	760835	Pectinase gene transcriptional regulator. [Escherichia coli] >gnl PID d1015936 Pectinase gene transcriptional regulator. [Escherichia coli] >gi 1787806 (AE000250) putative transcriptional regulator L YSR- type [Escherichia coli] >pir A64907 A64907 hypotheti	3	434	100	HE9BW44
117	761760	F45G2.10 [Caenorhabditis elegans] >sp O62252 O62252 F45G2.10 PROTEIN. Length = 160	3	527	61	HMWJF41
118	762520	B-myb protein (AA 1-700) [Homo sapiens] >pir S01991 S01991 transforming protein B-myb - human >sp P10244 MYBB_HUMAN MYB- RELATED PROTEIN B (B-MYB). Length = 700	77	520	100	HBJB76
119	764461		2	211		HOFMH95
120	764517	phosphomevalonate kinase [Homo sapiens] >sp Q15126 PMKA_HUMAN PHOSPHOMEVALONATE KINASE (EC 2.7.4.2) (PMKASE). {SUB 2-192} >gi 3445542 (AF026069) phosphomevalonate kinase [Homo sapiens] {SUB 33-192} Length = 192	260	877	100	HCGAA73

121	765132	clk1; putative [Homo sapiens] >pir S53641 S53641 protein kinase clk1 (EC 2.7.1.-) - human >sp P49759 CLK1_HUMAN PROTEIN KINASE CLK1 (EC 2.7.1.-) (CLK). Length = 484	gi 632964	1202	2251	99	99	HE9QA05
122	765667	(AF043250) mitochondrial outer membrane protein [Homo sapiens] >gi 3941347 (AF043253) mitochondrial outer membrane protein [Homo sapiens] >gi 4105703 (AF050154) DI9S1177E [Homo sapiens] >sp G3941342 G3941342 MITOCHONDRIAL OUTER MEMBRANE PROTEIN. >sp G3941	gi 3941342	144	1115	91	91	HCHOB54
123	767113	putative progesterone binding protein [Homo sapiens] >sp O00264 O00264 PUTATIVE PROGESTERONE BINDING PROTEIN. Length = 195	gnl P1D e3141 74	66	677	93	93	HNTMW26
124	767204	cytochrome P45011C4 [Oryctolagus cuniculus] >pir S20227 S20227 cytochrome P450 2C4 - rabbit (fragment) >sp Q29507 Q29507 CYTOCHROME P450 (EC 1.14.14.1) (FRAGMENT). Length = 145	gi 164933	3	581	43	61	HCHAN75
125	767400			2	1057			HSYBI74

126	767962	proteasome subunit C3 [Homo sapiens] >pir S15970 SNHUC3 multicatalytic endopeptidase complex (EC 3.4.99.46) chain C3 - human >sp P25787 PRC3_HUMAN PROTEASOME COMPONENT C3 (EC 3.4.99.46) (MACROPAIN SUBUNIT C3) (MULTICATALYTIC ENDOPEPTIDASE COMPLEX SUBUNIT (AB002086) p47 [Rattus norvegicus] >gn P D e294068 XY40 protein [Rattus norvegicus] >sp O35987 O35987 P47, COMPLETE CDS. Length = 370 adenine phosphoribosyltransferase [Homo sapiens] >gi 28819 adenine phosphoribosyltransferase (aprt) [Homo sapiens] >pir S06232 RTHUA adenine phosphoribosyltransferase (EC 2.4.2.7) - human >sp P07741 APT_HUMAN ADENINE PHOSPHORIBOSYLTRANSFERASE (EC 2.4.2.7)	gn P D d100 1115	3	722	100	100	HABAF63
127	768040		gn P D d102 2509	119	661	84	89	HSRDI53
128	769956		gi 178867	2	592	100	100	HUFC71
129	770133			958	1236			HUSAX93
130	770289	ALDH7 [Homo sapiens] >pir 38669 38669 ALDH7 - human >sp P43353 DHA7_HUMAN ALDEHYDE DEHYDROGENASE 7 (EC 1.2.1.5). >sp G601780 G601780 ALDH7. Length = 468	gi 601780	194	340	65	69	HCHAO38

131	771964	(AD000092) human RAD23A homolog [Homo sapiens] >gnl PID d1005299 HHR23A protein [Homo sapiens] >pir S44443 S44443 RAD23 protein homolog2 - human Length = 363	gil1905912	29	1165	76	76	HAMGD77
132	772582	B-myb protein (AA 1-700) [Homo sapiens] >pir S01991 S01991 transforming protein B-myb - human >sp P10244 MYBB_HUMAN MYB- RELATED PROTEIN B (B-MYB). Length = 700	gil29472	150	974	99	99	HYAAO51
133	773387	zinc finger protein [Homo sapiens] >pir I38620 I38620 zinc finger protein ZNF155 - human (fragment) Length = 139	gil495576	152	634	64	46	HAJBC78
134	773827	novel serine protease, PRSS11 [Homo sapiens] >gnl PID d1014012 serin protease with IGF-binding motif [Homo sapiens] >sp Q92743 Q92743 NOVEL SERINE PROTEASE. Length = 480	gnl PID e2751 86	3	1217	100	100	HKADJ15
135	774108	protein of unknown function [Homo sapiens] >pir C35826 C35826 hypothetical protein A, 13K - human >sp Q00994 HG74_HUMAN OVARIAN GRANULOSA CELL 13.0 KD PROTEIN HGR74. Length = 111	gil189379	303	623	75	75	HEGAC01

136	774636	glutathione transferase [Homo sapiens] >pir A39375 A39375 glutathione transferase (EC 2.5.1.18) class mu, GSTM2 - human >sp P28161 GTM2_HUMAN GLUTATHIONE S-TRANSFERASE MU 2 (EC 2.5.1.18) (GSTM2-2) (CLASS-MU). {SUB 2-218} >gnl PID e33921 glutathione transf	gil183301	61	747	98	98	HISDV78
137	775339	SWI/SNF complex 60 KDa subunit [Homo sapiens] >sp Q92924 Q92924 SWI/SNF COMPLEX 60 KDA SUBUNIT. Length = 435	gil1549243	3	320	98	100	HSIGB35
138	775582			448	705			HEPNB30
139	775779	(AJ000332) Glucosidase II [Homo sapiens] >sp Q14697 Q14697 GLUCOSIDASE II PRECURSOR (K1A A0088). >gnl PID d1008224 The ha1225 gene product is related to human alpha- glucosidase. [Homo sapiens] {SUB 2-944} Length = 944	gnl PID e3281 43	1	1695	98	98	HLWAS86
140	777809	cysteine-rich protein 2 [Homo sapiens] >gnl PID d1008288 ESP1/CRP2 [Homo sapiens] >pir G02090 G02090 cysteine-rich protein 2 - human >sp P52943 CRP2_HUMAN CYSTEINE- RICH PROTEIN 2 (CRP2) (ESPI PROTEIN). Length = 208 valyl-tRNA synthetase [Homo sapiens] >pir S17675 S17675 valine--tRNA ligase (EC 6.1.1.9) - human Length = 1265	gil1399028	202	681	99	100	HSPMB57
141	778927		gil31545	1843	3282	88	88	HIMVBW39

142	779262		1	288		HTENK29
143	779392		2	181		HE2FO87
144	780149	proteasome activator hPA28 suunit beta [Homo sapiens] >pir 53518 53518 proteasome activator hPA28 suunit beta - human >sp Q15129 Q15129 PROTEASOME ACTIVATOR HPA28 SUUNIT BETA. >sp G693763 G693763 PA28=REGULATORS OF THE 20 S PROTEASOME {PEPTIDE 15}. {SUB	233	955	93	HSPMF83
145	780583		8	607		HJIEOW04
146	780960		232	576		HOEBN65
147	781469	radixin [Homo sapiens] >pir A46127 A46127 radixin - human Length = 583	1	303	100	HN1RA25
148	781556		116	190		HOSAW82
149	781771		1	822		HE6EO05
150	782033	histone H2A [Gallus gallus] Length = 129	146	544	98	HULCC66
151	782105		606	1064	100	HKAKV16



152	782122	high density lipoprotein binding protein [Homo sapiens] >pir A44125 A44125 high density lipoprotein-binding protein, 110K - human >sp Q00341 HBP_HUMAN HIGH DENSITY LIPOPROTEIN BINDING PROTEIN (HDL-BINDING PROTEIN). >sp G1478463 G1478463 VIGILIN=KH PROTEIN	gi 183892	3	983	95	95	HSRAB32
153	783135	zinc finger protein [Homo sapiens] >sp O00488 O00488 ZINC FINGER PROTEIN. Length = 116	gn P D d 102 1201	3	500	97	99	HCHCB61
154	783245			3	341			HTSFV77
155	783247			95	391			HIBGMD18
156	783413	D9 splice variant 3 [Mus musculus] >sp O08695 O08695 D9 SPLICE VARIANT 3. Length = 169	gi 2071991	1	591	80	88	HIEBFR23
157	784407			45	185			HFKAA09
158	784548	nuclear RNA helicase (DEAD family) [Homo sapiens] >pir J37201 J37201 nuclear RNA helicase (DEAD family) BAT1 - human >sp Q13838 HE47_HUMAN PROBABLE ATP-DEPENDENT RNA HELICASE P47. >gi 2739119 (AF029061) BAT1 [Homo sapiens] {SUB 145-428} >gi 971677 express	gi 587146	676	1020	90	92	HSRFZ85

159	785075	KIAA0100 is a human counterpart of mouse e1 gene. [Homo sapiens] >sp Q14667 Q14667 KIAA0100 (HUMAN COUNTERPART OF MOUSE E1 GENE). Length = 2092	gnl PID d100 8477	72	1109	93	93	HDPFX40
160	785677	(AC004084) similar to DNA-DIRECTED RNA POLYMERASE II 13.3 KD POLYPEPTIDE; 98% similar to P5243 (PID:gi1710661) [Homo sapiens] >sp O43375 O43375 SIMILAR TO DNA-DIRECTED RNA POLYMERASE II 13.3 KD POLYPEPTIDE (FRAGMENT). Length = 105	gi 2822158	1	273	95	100	HBSA150
161	786238			2	994			HOVCA75
162	786389			3	1124			HLJDU61
163	786929	(A1224442) methyltransferase [Homo sapiens] >sp O43709 O43709 METHYLTRANSFERASE. Length = 220 PIPPin protein [Rattus norvegicus] >pir C4588 C4588 RNA-binding protein PIPPin - rat >sp Q63430 Q63430 PIPPIN PROTEIN. Length = 154	gnl PID c1253 426	123	404	86	95	HOINV27
164	786932		gi 1050754	2	490	76	87	HUSYH27
165	787078	HER2 receptor [Homo sapiens] >gi 553282 c-erb-2 protein [Homo sapiens] {SUB 737-1031} >gi 553332 HER-2/neu [Homo sapiens] {SUB 1-191} >gi 183989 HER2 receptor (AA at 3) [Homo sapiens] {SUB 740-910} >gi 182169 c-erb B2/neu protein [Homo sapiens] {SUB 1081-}	gi 306840	236	1114	79	79	HCHND12

166	787139			230	625			IHCBA06
167	787283			3	656			HFOYO96
168	788761	MAL3P6.24 [Plasmodium falciparum] >sp O77371 O77371 MAL3P6.24 PROTEIN. Length = 1017	gn PID e1331 909	2	700	36	60	HTXFK57
169	788988	(AF023611) Dim1p homolog [Homo sapiens] >sp O14834 O14834 DIM1P HOMOLOG. Length = 142	gi 2565275	70	417	98	98	HUSGH90
170	789092			2	400			H6EBE80
171	789298	(AF044311) gamma-synuclein [Homo sapiens] >gi 3642775 (AF017256) persyn [Homo sapiens] >gi 3642903 (AF037207) persyn [Homo sapiens] >sp O76070 O76070 PERSYN. Length = 127	gi 3347842	1	489	82	82	HTSFM20
172	789299			205	381			HBGDD91
173	789718			233	580			HIBGT30
174	789957	beta-hexosaminidase alpha chain [Homo sapiens] >pir A23561 AOHUBA beta-N-acetylhexosaminidase (EC 3.2.1.52) alpha chain precursor - human >sp P06865 HEXA_HUMAN BETA- HEXOSAMINIDASE ALPHA CHAIN PRECURSOR (EC 3.2.1.52) (N-ACETYL- BETA-GLUCOSAMINIDASE) (BETA-	gi 179458	750	1619	99	99	HIISEM44

175	789977	arginyl-tRNA synthetase, ArgRS [human, ataxia-telangiectasia patients, EBV-lymphoblastoid cells, Peptide, 659 aa] [Homo sapiens] >pir JC4365 JC4365 arginine--tRNA ligase (EC 6.1.1.19) - human Length = 659	bbs 173838	25	2019	94	95	HMEIU30
176	790285	HCG V [Homo sapiens] >sp O60927 O60927 HCG V. Length = 126	gi 3176438	44	391	85	85	HDPCH88
177	790509	human elongation factor-1-delta [Homo sapiens] >pir S34626 S34626 translation elongation factor eEF-1 delta chain - human >sp P29692 EF1D_HUMAN ELONGATION FACTOR 1-DELTA (EF-1-DELTA). Length = 281	gi 38522	227	1108	63	64	HIPMGB64
178	790775			950	1351			HJAAO21
179	790888	(AF036956) neuroblastoma apoptosis-related RNA binding protein [Homo sapiens] >sp G4104559 G4104559 NEUROBLASTOMA APOPTOSIS-RELATED RNA BINDING PROTEIN. Length = 490	gi 4104559	2	274	100	100	HE8QE19
180	791506			2	205			HOIFMB93
181	791649			3	359			HBGBH10
182	791802			165	695			HWLRH03

183	792002	ADP-ribosylation factor [Homo sapiens] >gi 2088529 ADP-ribosylation factor 5 [Homo sapiens] >gi 438870 ADP- ribosylation factor 5 [Rattus norvegicus] >gn P1D1014187 ARF5 [Mus musculus] >pir A23741 A23741 ADP-ribosylation factor 5 - human >pir JC4949 JC4	gi 178987	2	655	100	100	HHENT53
184	792291	see GenBank Accession Number U01184 for cDNA; similar to Drosophila melanogaster flit in GenBank Accession Number U01182 and Caenorhabditis elegans flit homolog in GenBank Accession Number U01183 [Homo sapiens] >sp Q13045 Q13045 FLIGHTLESS-1 PROTEIN HOMOL	gi 2138290	843	3329	96	96	IIDP1169
185	792371			3	665			IUSJW77
186	792660	(AF044773) breakpoint cluster region protein 1 [Homo sapiens] >sp O60558 O60558 BREAKPOINT CLUSTER REGION PROTEIN 1. Length = 138	gi 3002951	116	406	100	100	HCHMC26
187	792782			41	838			HTXJB38
188	792890	(AF001846) lymphoid phosphatase LyP1 [Homo sapiens] >sp G4100632 G4100632 LYMPHOID PHOSPHATASE LYPI. Length = 808	gi 4100632	2	994	90	90	HHESJ29
189	792931			1	576			HEGAW71

190	792943	myosin heavy chain kinase B [Dictyostelium discoideum] >sp P90648 KMH_B DICI_MYOSIN HEAVY CHAIN KINASE B (EC 2.7.1.129) (MHCK B). Length = 732	gi 1903458	3	1247	43	68	IIDPRZ79
191	793104			107	250			HKGAJ80
192	793445	desmoyokin - human (fragments) >sp Q09666 AHNK_HUMAN NEUROBLAST DIFFERENTIATION ASSOCIATED PROTEIN AHNK (DESMOYOKIN) (FRAGMENTS). >gi 178281 AHNAK_nucleoprotein [Homo sapiens] {SUB 1-1683} >gi 897824 AHNAK gene product [Homo sapiens] {SUB 1684-2960} Leng	pir A45259 A 45259	1	723	92	92	HDTEJ86
193	793446			25	255			HIIBGY94
194	793639	(AF044959) NADH:ubiquinone oxidoreductase NDUFS6 subunit [Homo sapiens] >sp O75380 NUMM_HUMAN NADH-UBIQUINONE OXIDOREDUCTASE 13 KD-A SUBUNIT PRECURSOR (EC 1.6.5.3) (EC 1.6.99.3) (COMPLEX I-13KD-A) (CI-13KD-A). Length = 124	gi 3348137	1	411	100	100	HLJB172
195	794213	100 kDa protein [Rattus norvegicus] >pir S22659 S22659_hypothetical protein, 100K - rat >sp Q62671 100K_RAT 100 KD PROTEIN (EC 6.3.2.-). Length = 889	gi 55535	326	691	93	95	HLWCN67
196	795858			1020	1205			HLVDY53

197	795955	c-myc binding protein [Homo sapiens] >sp Q99471 MM1_HUMAN C-MYC BINDING PROTEIN MM-1. >sp D1014706 D1014706 C-MYC BINDING PROTEIN. Length = 167	gnl PID d101 4706	31	507	100	100	100	HUSXX36
198	796359	ribosomal protein L7a large subunit [Homo sapiens] >gi 34203 L7a protein [Homo sapiens] >gi 35512 PLA-X polypeptide [Homo sapiens] >gi 36647 ribosomal protein L7a [Homo sapiens] >gi 56956 ribosomal protein L7a (AA 1-266) [Rattus rattus] >pir S19717 R5HU7A	gi 337495	19	297	100	100	100	HOFNW79
199	796555	DJ366N23.3 (KIAA0173 AND TUBULIN- TYROSINE LIGASE LIKE) (FRAGMENT). Length = 278	sp O75653 O7 5653	1	1086	44	62	62	HLWEW04
200	796675	PEG1/MEST [Homo sapiens] >sp O15007 O15007 PEG1/MEST GENE MRNA. Length = 335	gnl PID e3070 37	44	1027	100	100	100	HSICR25
201	796743	(AF022229) translation initiation factor 6 [Homo sapiens] >gnl PID e304603 b4 integrin interactor [Homo sapiens] >gi 3335506 (AF047433) b(2)gen homolog [Homo sapiens] >sp P56537 IF6_HUMAN EUKARYOTIC TRANSLATION INITIATION FACTOR 6 (EIF-6) (B4 INTEGRIN INT	gi 2809383	30	842	100	100	100	H6EDU12
202	796792			198	461				HDTII72
203	799668			166	303				HODBC01
204	799669			2	310				HOGAV29

205	799673		2	310		HOFMN53
206	799674		130	1044		HCHM160
207	799678	ribosomal protein L18a [Homo sapiens] >gi 3702270 (AC005796) ribosomal protein L18a [Homo sapiens] >gnl PID d1029536 (AB007175) ribosomal protein L18a [Homo sapiens] {SUB 111-176} Length = 176	40	345	98	HOFNL25
208	799728		3	179		HIBGIG75
209	799748		1	660		HCTIMQ24
210	799760	o361 [Escherichia coli] >gi 1790125 (AE000446)orf, hypothetical protein [Escherichia coli] >pir C65171 C65171 hypothetical 41.0 kD protein in ibpA-gyrB intergenic region - Escherichia coli (strain K-12) Length = 361	1	357	99	HIBGBIF66
211	799805		2	118		HIBGDA22
212	800296	CDC37 homolog [Homo sapiens] >gi 1375485 CDC37 homolog [Homo sapiens] >pir G02313 G02313 CDC37 homolog - human >sp Q16543 Q16543 CDC37 HOMOLOG. Length = 378	2	802	89	HIDABE68



213	800327	ADP-ribosylation factor-like protein 2 [Homo sapiens] >pir A48259 A48259 ADP- ribosylation factor-like 2 - human >sp P36404 ARL2_HUMAN ADP- RIBOSYLATION_FACTOR-LIKE PROTEIN 2. >sp G425655 G425655 ARL2=ADP-RIBOSYLATION FACTOR HOMOLOG. Length = 184	gj 3009501	25	645	99	99	HCHPG41
214	800816			115	351			HODCV09
215	800835	(AF071538) Ets transcription factor PDEF [Homo sapiens] >sp G4007418 G4007418 ETS TRANSCRIPTION FACTOR PDEF. Length = 335	gj 4007418	3	881	96	96	HETJP29
216	805429	RanGAP1 [Homo sapiens] >pir JC5300 JC5300 Ran GTPase activator 1 - human Length = 587	gj 575268	3	683	90	90	HKABS06
217	805458	(AF044221) HCG-1 protein [Homo sapiens] >sp G4105252 G4105252 HCG-1 PROTEIN. Length = 117	gj 4105252	745	1122	100	100	HDQEV55
218	805478			60	644			HDQGR35
219	805805	19 kDa subunit of NADH:ubiquinone oxidoreductase complex (complex I) [Bos taurus] >pir S16208 S16208 NADH dehydrogenase (ubiquinone) (EC 1.6.5.3) 19K chain - bovine >sp P42029 NUPM_BOVIN NADH- UBIQUINONE OXIDOREDUCTASE 19 KD SUBUNIT (EC 1.6.5.3) (EC 1.6.99	gj 599681	2	478	87	90	HOFMH12
220	806486			3	62			HFXJC33

221	806498		518	1741		HIBCA25	
222	806819	acidic ribosomal phosphoprotein (P0) [Homo sapiens] >gi 2935618 (AC004263) 60S ACIDIC RIBOSOMAL PROTEIN; match to P05388 (PID:gi 33041) [Homo sapiens] >pir A27125 R5HUP0 acidic ribosomal protein P0 - human >sp D1026785 D1026785 RIBOSOMAL PROTEIN P0 (FRAGME	gi 190232	3	866	84	HOFAC09
223	810870	thrombospondin-4 [Homo sapiens] >pir A55710 TSHUP4 thrombospondin 4 precursor - human Length = 961	gi 311626	2	1333	99	HBOEB83
224	811730			2	979		HCHPI26
225	813025	heat shock protein 86 [Homo sapiens] >sp Q14568 Q14568 HEAT SHOCK PROTEIN 86 (FRAGMENT). Length = 312	gi 292162	106	492	88	HOFMD78
226	813233	co-beta glucosidase precursor [Homo sapiens] >gi 337762 prosaposin [Homo sapiens] >gi 337756 sphingolipid activator precursor [Homo sapiens] Length = 524	gi 183231	1	468	81	HOFMF17
227	813262			1	345		HFKCA89
228	815637	(AC004003) serine/threonine kinase RICK; match to protein AF027706 (PID:gi 3123887) and mRNA AF027706 (NID:gi 3123886) [Homo sapiens] >gi 3290172 (AF064824) CARD-containing ICE associated kinase [Homo sapiens] >gi 3342910 (AF078530) receptor	gi 3264574	3	461	92	HNHDS66

## interacting prote

229	815853	calcyphosine [Homo sapiens] >gi3075376 (AC004602) CAYP_HUMAN; RD25 [Homo sapiens] >sp Q13938 CAYP_HUMAN CALCYPHOSINE; Length = 189	gnl PID e2458 72	8	667	100	100	HLHAY85
230	815999	S100 calcium-binding protein A13 (S100A13) [Homo sapiens] >pir JC5064 JC5064 S-100 calcium-binding protein A13 - human Length = 98	gnl PID e2682 53	68	421	42	70	HKABX07
231	823427			1	927			HTLGL50
232	823704	(AC004770) BC269730_2 [Homo sapiens] >sp O60427 O60427 BC269730_2. Length = 444	gi 3169158	3	860	67	80	HDABC49
233	824798			307	858			HDQ GK75
234	825018			2	1924			HETIS29
235	825076	Whole ORF continues from bp19 (right after 'tag') to bp1596 ('tag'); similar to chinese hamster phosphatidylserine synthase. [Homo sapiens] Length = 473	gnl PID d100 4031	2	1549	92	92	HE9PJ48

236	825787	EXT2 [Homo sapiens] >gi 1621113 hereditary multiple exostoses gene 2 protein [Homo sapiens] >gi 1519605 multiple exostosis 2 [Homo sapiens] >sp Q93063 EXT2_HUMAN EXOSTOSIN-2 (PUTATIVE TUMOUR SUPPRESSOR PROTEIN EXT2) (MULTIPLE EXOSTOSES PROTEIN 2). Length =	gi 1518042	305	2293	100	100	11EONV84
237	826116	BETA CRYSTALLIN S (GAMMA CRYSTALLIN S). >gi 557548 crystallin [Homo sapiens] {SUB 19-106} Length = 177	sp P22914 CR BS_HUMAN	392	682	86	87	HJAE27
238	826147	neural specific protein CRMP-2 [Bos taurus] >sp O02675 DPY2_BOVIN DIHYDROPYRIMIDINASE RELATED PROTEIN-2 (DRP-2) (NEURAL SPECIFIC PROTEIN NSP60). Length = 572	gi 1916227	3	503	98	98	ICEPT06
239	827020	(AF027954) Bcl-2-related ovarian killer protein [Rattus norvegicus] >gi 2689660 (AF027707) apoptosis activator Mid [Mus musculus] >sp O35425 O35425 BCL-2- RELATED OVARIAN KILLER PROTEIN. Length = 213	gi 2645560	12	539	95	97	HHFHE17
240	827586	calmodulin [Plasmodium falciparum] >gi 160128 calmodulin [Plasmodium falciparum] >pir B45594 MCZQF calmodulin - Plasmodium falciparum >sp P24044 CALM_PLAFA CALMODULIN. Length = 149	gi 385234	85	495	49	76	HCHMW40

241	827732	alternate name ygiG; ORF_f123 [Escherichia coli] >gi 1789438 (AE000387) putative kinase [Escherichia coli] >pir H65093 H65093 ygiG protein - Escherichia coli (strain K-12) >sp P31055 FOLB_ECOLI PROBABLE DIHYDRONEOPTERIN ALDOLASE (EC 4.1.2.25) (DHNA). {SUB	gi 882580	181	282	91	95	HBGDE81
242	827735			541	708			HHEDU22
243	827740			716	838			HBNA17
244	827808			86	1657			HMELR44
245	828251	(AB016869) p70 ribosomal S6 kinase beta [Homo sapiens] >sp D1035383 D1035383 P70 RIBOSOMAL S6 KINASE BETA. Length = 495	gn P D d 103 5383	134	949	91	91	HINGOL64
246	828357			1	768			HKIYP61
247	828449			1	723			HBXCZ22
248	828612	syntaxin 5 [Homo sapiens] >pir G01817 G01817 syntaxin 5 - human Length = 301	gi 886071	68	460	100	100	IINHMY58
249	828647	laminin beta 2 chain [Homo sapiens] >sp P55268 LMB2_HUMAN LAMININ BETA-2 CHAIN PRECURSOR (S- LAMININ). Length = 1798	gn P D e 2132 86	299	2254	85	85	HRABB47

250	828698	galactokinase [Homo sapiens] >gi 1929895 galactokinase [Homo sapiens] >sp P51570 GAL1_HUMAN GALACTOKINASE 1 (EC 2.7.1.6). >gi 3603423 (AF084935) galactokinase [Homo sapiens] {SUB 1-264} Length = 392	gi 1002507	3	1220	83	83	HKG AU37
251	828962	secretory protein [Homo sapiens] >gi 940946 intestinal trefoil factor [Homo sapiens] >pir A48284 A48284 intestinal trefoil factor 3 precursor - human >sp Q07654 ITF_HUMAN INTESTINAL TREFOIL FACTOR PRECURSOR (HP1.B). Length = 80	gi 402483	2	259	78	78	HCHMR52
252	828982	unnamed protein product [unidentified] >gi 189500 p62 [Homo sapiens] >pir A38219 A38219 GAP-associated tyrosine phosphoprotein p62 - human >sp Q07666 Q07666 GAP-ASSOCIATED TYROSINE PHOSPHOPROTEIN P62. >gnl PID e 259626 unnamed protein product [unidentified]	gnl PID e 259 622	1	1176	85	85	HE9PC52
253	829282			289	828			HCHOB95
254	829368			279	512			HWGAA79
255	829751			2	418			HCHMB33
256	829773	(AF109906) G9A [Mus musculus] >sp G3986768 G3986768 G9A. Length = 1000	gi 3986768	26	862	97	98	HMWBV67

257	829934	precursor polypeptide (AA -21 to 782) [Homo sapiens] >pir A35954 A35954 endoplasmin precursor - human >sp P14625 ENPL_HUMAN ENDOPLASMIN PRECURSOR (94 KD GLUCOSE-REGULATED PROTEIN) (GRP94) (GP96 HOMOLOG) (TUMOR REJECTION ANTIGEN 1). Length = 803	gi 37261	1142	2356	94	94	HFIJJ68
258	829942	dynamitin [Homo sapiens] >sp Q13561 DYNC_HUMAN DYNACTIN, 50 KD ISOFORM (50 KD DYNEIN-ASSOCIATED POLYPEPTIDE) (DYNAMITIN). Length = 406	gi 1255188	15	1409	85	85	HUFBF69
259	829951			119	262			HIBGBA32
260	830173	death associated protein 5 [Homo sapiens] >sp O60877 O60877 DEATH ASSOCIATED PROTEIN 5. Length = 907	gi P1D1e1298 888	51	2870	90	90	HETJX39
261	830200			3	638			HIBGMF83
262	830365	mevalonate pyrophosphate decarboxylase [Homo sapiens] >sp P53602 ER19_HUMAN DIPHOSPHOMEVALONATE DECARBOXYLASE (EC 4.1.1.33) (MEVALONATE PYROPHOSPHATE DECARBOXYLASE). Length = 400	gi 1235682	56	1291	95	95	HUSJG21
263	830456			215	397			HCFCBN01

264	830549	guanine nucleotide-binding regulatory protein-beta-2 subunit [Homo sapiens] >gi 339935 transducin beta-2 subunit [Homo sapiens] >gi 3135310 (AF053356) GNB2 [Homo sapiens] >pir B26617 RGHUB2 GTP-binding regulatory protein beta-2 chain - human >sp P11016 GB	gi 386751	1	729	100	100	HDPM12
265	830602			24	461			HTLD182
266	830610	zyxin [Homo sapiens] >gnl P1D1e223417 zyxin [Homo sapiens] >pir G02845 G02845 zyxin - human Length = 572	gnl P1D1e2182 60	956	1855	94	94	HDPRN35
267	830644	(AF104260) hiwi [Homo sapiens] >sp G4038413 G4038413 HIWI (FRAGMENT). Length = 523	gi 4038413	2	391	99	99	HTTEU95
268	830707			3	623			HETC114
269	830709			2	304			HSSGN20
270	830733			540	725			HSNAD86
271	830768	carboxylesterase hCE-2 [Homo sapiens] >sp Q16859 Q16859 CARBOXYLESTERASE (EC 3.1.1.1) (AL1-ESTERASE) (B-ESTERASE) (MONOBUTYRASE) (COCAINE ESTERASE) (PROCAINE ESTERASE) (METHYLBUTYRASE). Length = 550	gi 407780	623	2269	99	99	IIDPF44
272	830855			1	465			HJPCE06



273	830949		2457	2903		HCE5J35
274	830965		139	792		IIOLICAD1
275	830973		354	557		HRD1.42
276	830979	THIOREDOXIN REDUCTASE 2. Length = 526	753	1454	81	HOGCC93
277	830989	La protein [Homo sapiens] >gi 36415 ribonucleoprotein SS-B/La (AA 1-408) [Homo sapiens] >pir A31888/A31888 ribonucleoprotein La - human >sp P05455 LA_HUMAN.LUPUS LA PROTEIN (SJOEGREN SYNDROME TYPE B ANTIGEN (SS-B)) (LA RIBONUCLEOPROTEIN) (LA AUTOANTIGEN).	3	1382	87	IIDQFZ49
278	831134		2	241		HBXEB46
279	831200		3	773		HADXB20
280	831260		892	1095		HLWBR58
281	831531	transcription factor [Homo sapiens] >gi 37058 IIB protein [Homo sapiens] >pir S17654 TWHU2B transcription initiation factor IIB - human >bbs 112738 S300-II, TF1IB=transcription factor [human, Peptide Partial, 311 aa] [Homo sapiens] {SUB 6-316} Length = 31	93	1172	95	HHPGX85
282	831665		2	1093		HSKDH81

283	831724			1	468		HFEBQ94
284	831884	(AF034800) liprin-alpha3 [Homo sapiens] >sp G3309535 G3309535 LIPRIN- ALPHA3 (FRAGMENT). Length = 443	gij 3309535	20	469	90	HDTGO74
285	831897	laminin B1 [Homo sapiens] >gij 186876 laminin B1 [Homo sapiens] >gij 186913 laminin B1 [Homo sapiens] >pir S13547 MMHUB1 laminin chain B1 precursor - human >sp P07942 LMB1_HUMAN LAMININ BETA-1 CHAIN PRECURSOR (LAMININ B1 CHAIN). Length = 1786	gij 186837	1	1581	92	HSKHV84
286	831922			499	684		HDQIB68
287	831963			188	319		HDPGS84
288	832074	gluconate kinase [Escherichia coli] >gij 1790719 (AE000497) gluconate kinase, thermosensitive glucokinase [Escherichia coli] >pir S56494 S56494 gluconokinase (EC 2.7.1.12) gntV - Escherichia coli >sp P39208 GNTV_ECOLI THERMOSENSITIVE GLUCONOKINASE (EC 2.7.	gij 537110	1	579	42	HCRNT71
289	832266			71	433		HNGJU70
290	832309			1891	2226		IIBJDT21
291	832342	fatty acid amide hydrolase [Homo sapiens] >sp O00519 O00519 FATTY ACID AMIDE HYDROLASE. Length = 579	gij 2149156	9	224	97	HBGDP82
						100	

292	832351	unknown product specific to adipose tissue [Homo sapiens] >sp Q15847 Q15847 HYPOTHETICAL 7.9 KD PROTEIN. Length = 76	gnl PID d100 8821	47	298	68	68	HFABE30
293	832352	unknown product specific to adipose tissue [Homo sapiens] >sp Q15847 Q15847 HYPOTHETICAL 7.9 KD PROTEIN. Length = 76	gnl PID d100 8821	89	277	94	92	HIOEKX93
294	832434	Cks1 protein homologue [Homo sapiens] >pir A36670 A36670 protein kinase cdc2 complex subunit Cks1 - human >sp P33551 CKS1_HUMAN CYCLIN- DEPENDENT KINASES REGULATORY SUBUNIT 1 (CKS-1). Length = 79	gi 29977	78	335	100	100	HFNAB43
295	832490	growth arrest and DNA-damage-inducible protein [Homo sapiens] >gi 403128 [Human gadd45 gene, complete cds.], gene product [Homo sapiens] >pir A39617 A39617 DNA-damage-inducible protein gadd45 - human >sp P24522 GA45_HUMAN GROWTH ARREST AND DNA- DAMAGE-INDU	gi 182940	220	798	100	98	HKAKL21
296	832573			30	629			HCHOY13
297	832580	pS2 protein [Homo sapiens] >gi 35707 pS2 precursor [Homo sapiens] >gnl PID e223341 pS2 [Homo sapiens] >pir A26667 A26667 pS2 protein precursor - human >gi 182204 estrogen receptor [Homo sapiens] (SUB 2-84) Length = 84	gi 35718	45	362	100	100	H2LAR67

298	833394		274	588		HBCMC47
299	835355	(AF060567) sushi-repeat protein [Homo sapiens] >sp O60687 O60687 SUSHI-REPEAT PROTEIN. Length = 465	3	1295	99	HUSAU05
300	835497	(AJ006064) coronin-like protein [Rattus norvegicus] >sp O89046 O89046 CORONIN-LIKE PROTEIN. Length = 484	334	1584	96	HLDD571
301	835728		2	871		HODAK21
302	835978		643	2019		ITLLEB03
303	836091	PDC-E2 precursor (AA -54 to 561) [Homo sapiens] >pir S01783 XXHU dihydroipoamide S-acetyltransferase (EC 2.3.1.12) precursor - human (fragment >gi 345030 Human 70kd mitochondrial antigen of PBC [unidentified] {SUB 179-500} >sp G254062 G254062 PYRUVATE D	546	2114	99	IICCBW86
304	836274	Id4 [Homo sapiens] >gnl PID e266418 helix-loop-helix protein [Homo sapiens] >gnl PID e1359205 (AL022726) dJ625H18.1 (ID4 Helix-loop-helix DNA binding protein) [Homo sapiens] >gnl PID e266418 helix-loop-helix protein [Homo sapiens] >pir G01855 G01855 Id4 -	2	334	98	HCLBP52

305	836731	(AF075599) ubiquitin conjugating enzyme 12 [Homo sapiens] >gnl PID d1034111 (AB012191) Nedd8-conjugating enzyme hUbc12 [Homo sapiens] >sp O76069 O76069 UBIQUITIN-CONJUGATING ENZYME E2 (EC 6.3.2.19) (UBIQUITIN-PROTEIN LIGASE) (UBIQUITIN CARRIER PROTEIN). L	gi 3309661	2	571	100	100	HFXAZ01
306	838014	prolyl 4-hydroxylase alpha (II) subunit [Homo sapiens] >sp O15460 O15460 PROLYL 4-HYDROXYLASE ALPHA (II) SUBUNIT (II). Length = 535	gi 2439985	3	1574	99	99	HTEHY24
307	838874			271	546			HFEZ63
308	839120	peptide transporter [Homo sapiens] >pir S13427 A41538 ATP-binding cassette transporter TAP1 - human >gi 34636 ABC-transporter [Homo sapiens] {SUB 61-808} >gi 930122 Y3 gene product [Homo sapiens] {SUB 183-612} Length = 808	gi 36061	100	2169	90	90	HNIDY03
309	839611			548	793			HAMF154
310	840138	start position 1 [Homo sapiens] >sp E1335356 E1335356 ASMTL PROTEIN. >gnl PID e1335357 start position 2 [Homo sapiens] {SUB 59-629} Length = 629	gnl PID e1335356	1	1800	92	93	HFIHW86

311	840616	Homology with Squid retinal-binding protein (PIR Acc. No. A53057) [Caenorhabditis elegans] >sp Q22467 Q22467.T13H5.2.PROTEIN. Length = 1254	gnl PID e1349397	3	1607	73	86	HMSCY51
312	840780	unknown [Saccharomyces cerevisiae] >pir S58704 S58704.probable membrane protein Y1L003w - yeast (Saccharomyces cerevisiae) >gij S58401.incomplete orf, len: 160, CAl: 0.09 similar to MRP_ECOL1 P21590.39.9 KD PROTEIN [Saccharomyces cerevisiae] {SUB 1-158} >g	gij 763343	17	880	57	80	H6EDY61
313	840857	(AF071059) zinc finger RNA binding protein [Mus musculus] >sp O88532 O88532.ZINC FINGER RNA BINDING PROTEIN. Length = 1052	gij 3293537	459	2669	94	94	HLHDQ83
314	840862	cysteine-rich intestinal protein [Homo sapiens] >pir G02666 G02666.cysteine-rich protein 1 - human Length = 77	gij 1381638	36	353	100	100	HEPAP58
315	840864			407	1096			HTLHY48
316	840936	homologous to Swiss-Prot accession number P16371 [Homo sapiens] >gij 3850562 (AC005944) GRG_HUMAN; ESP1 PROTEIN; AMINO ENHANCER OF SPLIT; AES-1/AES-2; gp130 associated protein GAM [Homo sapiens] >pir G01236 G01236.enhancer of split m9/m10 (groucho protein)	gij 435425	3	668	79	79	HOENU32

317	840938	carbonyl reductase [Sus scrofa] >pir JN0703 JN0703 carbonyl reductase (NADPH) (EC 1.1.1.184) - pig >sp Q29529 CBR2_PIG LUNG CARBONYL REDUCTASE [NADPH] (EC 1.1.1.184) (NADPH-DEPENDENT CARBONYL REDUCTASE) (LCR). Length = 244	gnl PID d100 4479	2	745	65	76	HMCA175
318	841884			677	1324			HLQBI45
319	842241	(AJ009698) embigin protein [Rattus norvegicus] >sp O88775 O88775 EMBIGIN PROTEIN PRECURSOR. Length = 328	gnl PID e1312 986	2	952	60	75	HOFMD52
320	843712			2	202			HSSGR77
321	844040	ribosomal protein L11 [Caenorhabditis elegans] >pir S27795 S27795 ribosomal protein L11 homolog - Caenorhabditis elegans Length = 195	gij 56201	75	500	42	64	HPTGB84
322	844336	(AB009462) LDL receptor related protein 105 [Homo sapiens] >sp O75074 O75074 LDL RECEPTOR RELATED PROTEIN 105. Length = 770	gnl PID d103 3292	831	2285	68	75	HWMFE21
323	844612	collagen binding protein 2 [Homo sapiens] >pir 52968 52968 collagen-2 - human >sp P50454 CBP2_HUMAN COLLAGEN- BINDING PROTEIN 2 PRECURSOR (COLLIGIN 2). Length = 418	gnl PID d101 2496	528	1466	96	97	HOFME75
324	844617			556	735			HMVC236

325	845251	LIV-1 protein [Homo sapiens] >pir G02273 G02273 LIV-1 protein - human >sp Q13433 Q13433 ESTROGEN REGULATED LIV-1 PROTEIN. Length = 752	gi 1256001	23	634	49	67	HBGBB42
326	845764			2	244			HULCF61
327	846187	ATPase alpha subunit (aa 1-1023) [Homo sapiens] >gnl PID d1000505 Na,K-ATPase alpha-subunit [Homo sapiens] >pir A24414 A24414 Na+/K+-exchanging ATPase (EC 3.6.1.37) alpha-1 chain - human >sp P05023 ATN1_HUMAN SODIUM/POTASSIUM- TRANSPORTING ATPASE ALPHA-1 C	gi 28927	151	2403	92	92	HIDPLV27
328	HBGDH47R			167	241			HHGDI147
329	HHENQ86R			2	112			HHENQ86
330	HBGBH23R	(AE000161) bacteriophage lambda endopeptidase homolog [Escherichia coli] >pir B64788 B64788 bacteriophage lambda endopeptidase homolog (EC 3.4.-.-) - Escherichia coli (strain K-12) >sp P75719 ENPP_ECOLI PUTATIVE ENDOPEPTIDASE (EC 3.4.-.-). Length = 153	gi 1786769	1	213	92	92	HBGBH23
331	HANGA53R	(AF013214) acidic ribosomal phosphoprotein PO [Bos taurus] Length = 302	gi 2293577	76	402	80	84	HANGA53



332	HBIMC29R	(AF035959) type-2 phosphatidic acid phosphatase-gamma; phosphatidate phosphohydrolase; phospholipid phosphatase [Homo sapiens] >gij3025880 (AF056083) phosphatidic acid phosphatase type 2 [Homo sapiens] >gij2911498 (AF047760) phosphatidic acid phosphohydro (AF061340) F1 ATPase subunit 6 [Artibeus jamaicensis] Length = 226 (AF070447) barrier-to-autointegration factor [Homo sapiens] >sp O75531 O75531 BARRIER-TO-AUTOINTEGRATION FACTOR. Length = 89	gij3123896	3	317	96	96	HBIMC29
333	HOFAB89R		gij4164480	86	268	67	82	HOFAB89
334	HAHCP93R		gij3220255	116	289	69	76	HAHCP93
335	HBGAA76R			14	232			HBGAA76
336	HBGBT12R	A (DNA packaging,641) [Bacteriophage lambda] >pir D04333 VBPAL DNA-packaging protein A - phage lambda Length = 641	gij215106	2	349	95	95	HBGBT12
337	HGBBH53R	Actin [Drosophila melanogaster] >pir S14851 S14851 actin - fruit fly (Drosophila melanogaster) >sp Q24228 Q24228 ACTIN. Length = 100	gij7550	2	445	93	97	HGBBH53

338	HTXPI29R	aldolase A (EC 4.1.3.13) [Homo sapiens] >gi 28597 aldolase A (AA 1-364) [Homo sapiens] >pir S14084 ADHUA fructose-bisphosphate aldolase (EC 4.1.2.13) A - human >sp P04075 ALFA_HUMAN FRUCTOSE-BISPHOSPHATE ALDOLASE A (EC 4.1.2.13) (MUSCLE-TYPE ALDOLASE). {S	gi 178351	1	453	86	86	HTXPI29
339	HOFMG33R	ATPase [Equus caballus] >sp P48662 ATP6_HORSE ATP SYNTHASE A CHAIN (EC 3.6.1.34) (PROTEIN 6). Length = 226	gi 577577	28	309	57	62	HOFMG33
340	HCGAC11R			1	345			HCGAC11
341	HCIAC54R			37	168			HCIAC54
342	HBGAA54R			1	282			HBGAA54
343	HAOMC34R	calpactin I heavy chain (p36) [Bos taurus] >pir A03081 LUBO36 annexin II - bovine >sp P04272 ANX2_BOVIN ANNEXIN II (LIPOCORTIN II) (CALPACTIN I HEAVY CHAIN) (CHROMOBINDIN 8) (P36) (PROTEIN I) (PLACENTAL ANTICOAGULANT PROTEIN IV) (PAP-IV). {SUB 2-339} Leng	gi 162779	2	115	73	80	HAOMC34
344	H2LAU88R	copine I [Homo sapiens] >sp Q99829 Q99829 COPINE I. Length = 537	gi 1791257	1	576	95	95	H2LAU88
345	HDPJR77R	DNA topoisomerase II [Homo sapiens] >gi 38325 DNA topoisomerase II [Homo sapiens] {SUB 448-681} Length = 1031	gi 288565	3	311	100	100	HDPJR77

346	HTTIO41R	docking protein [Homo sapiens] >pir A29440 A29440 signal recognition particle receptor - human Length = 638	gj 30866	90	404	94	95	HTTIO41
347	H2CBU29R	electron transport flavoprotein [Homo sapiens] >pir A31998 A31998 electron transfer flavoprotein alpha chain precursor - human >sp P13804 ETFA_HUMAN ELECTRON TRANSFER FLAVOPROTEIN ALPHA-SUBUNIT PRECURSOR (ALPHA-ETF). >gnl P1D1e1331769 (AJ224002) electron	gj 182251	2	442	100	100	H2CBU29
348	HBMVA11R	GARS protein [Homo sapiens] >sp Q15374 Q15374 GARS PROTEIN. Length = 433	gnl P1D1d100 7383	1	108	81	84	HBMVA11
349	HDPUL86R	GC kinase [Homo sapiens] >pir A53714 A53714 protein kinase (EC 2.7.1.37) BL44 - human >sp Q12851 Q12851 GC KINASE. Length = 819	gj 531820	3	317	64	65	HDPUL86
350	HTXNT16R	GTP-binding protein [Homo sapiens] >gj 577779 GTP-binding protein [Homo sapiens] >pir A55014 A55014 GTP-binding protein - human >sp P55039 DRG2_HUMAN DEVELOPMENTALLY REGULATED GTP-BINDING PROTEIN DRG2. Length = 364	gj 577779	2	463	100	100	HTXNT16
351	HBGAA13R	H (tail component;853) [Bacteriophage lambda] >pir G43008 TLBPHL minor tail protein precursor H - phage lambda Length = 853	gj 215120	1	267	97	97	HBGAA13

352	HLXNA54R	heat shock protein HSP27 [Homo sapiens] >gi 433598.28.kDa.heat shock protein [Homo sapiens] >gi 1913885.heat shock protein [Homo sapiens] >pir S12102 HHHU27.heat shock protein 27 - human >sp G248440 G248440.28.KDA HEAT SHOCK PROTEIN HOMOLOG FRAGMENT 2. {S	gi 32478	2	256	98	98	HLXNA54
353	HCHOH37R	Hep27 protein [Homo sapiens] >pir S66665 S66665.nuclear protein Hep27 - human >sp Q13268 HE27_HUMAN HEP27 PROTEIN (PROTEIN D), {SUB 24-280} Length = 280	gi 1079566	337	564	75	81	HCHOH37
354	H2LAX93R	histone H2B [Gallus gallus] >gi 63434 histone H2B [Gallus gallus] >gi 63452 histone H2B (AA 1 - 126) [Gallus gallus] >gi 63456.histone H2B (AA 1 - 126) [Gallus gallus] >gi 63458.histone H2B [Gallus gallus] >gi 63460.histone H2B (AA 1 - 126) [Gallus gallus] homologue to elongation factor 1-gamma from A.salina [Homo sapiens] >gi 31104 elongation factor-1-gamma [Homo sapiens] >pir S22655 S22655.translation elongation factor eEF-1 gamma chain - human >sp P26641 EF1G_HUMAN ELONGATION FACTOR 1-GAMMA (EF- 1-GAMMA).	gi 211845	191	505	89	96	H2LAX93
355	HWAFW10R		gi 31102	3	434	98	98	HWAFW10

356	HBNAB19R	human complement C1r [Homo sapiens] >pir A24170 CIHURB complement subcomponent C1r (EC 3.4.21.41) precursor - human >sp P00736 C1R_HUMAN COMPLEMENT C1R COMPONENT PRECURSOR (EC 3.4.21.41). Length = 705	gij179644	2	193	98	98	HBNAB19
357	HBGDD17R	hypothetical protein [Escherichia coli] >gij1786774 (AE000161)orf, hypothetical protein [Escherichia coli] >pir G64788 G64788 hypothetical protein b0561 - Escherichia coli (strain K-12) Length = 247	gij1778474	1	207	98	98	HBGDD17
358	HBIAB72R	hypoxanthine phosphoribosyltransferase [Sus scrofa] >sp P79306 P79306 HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE (FRAGMENT). Length = 85	gn P D e2919 69	2	169	81	86	HBIAB72
359	HFIIEH41R	interferon-gamma induced protein [Homo sapiens] >pir I54501 I54501 interferon gamma-induced protein IFI 16 - human >sp Q16666 IFI16_HUMAN GAMMA- INTERFERON-INDUCIBLE PROTEIN IFI-16 (INTERFERON-INDUCIBLE MYELOID DIFFERENTIATION TRANSCRIPTIONAL ACTIVATOR). Le	gij184569	5	406	96	97	HFIIEH41
360	H2CBB43R	J (tail: host specificity; 1132) [Bacteriophage lambda] >pir D43009 QSBPL host specificity protein J - phage lambda Length = 1132	gij215125	2	400	99	99	H2CBB43

361	H2CBQ77R	J (tail:host specificity;1132) [Bacteriophage lambda] >pir[D43009]QSBPL host specificity protein J - phage lambda Length = 1132	gi 215125	3	272	97	97	H2CBQ77
362	HATAO24R	J (tail:host specificity;1132) [Bacteriophage lambda] >pir[D43009]QSBPL host specificity protein J - phage lambda Length = 1132	gi 215125	2	247	71	71	HATAO24
363	HOEMK06R	K (tail component;199) [Bacteriophage lambda] >pir[H43009]TJBPKL tail assembly protein K - phage lambda Length = 199	gi 215123	3	149	97	97	HOEMK06
364	HADCH03R	mitochondrial acetoacetyl-CoA thiolase precursor [Homo sapiens] Length = 427	gn PIDJ1014983	2	256	83	83	HADCH03
365	HCHAG30R	Mta1 [Rattus norvegicus] >pir[A54766]A54766 metastasis-associated protein mta-1 - rat >sp Q62599 MTA1_RAT METASTASIS-ASSOCIATED PROTEIN MTA1. Length = 703	gi 595253	2	271	92	92	HCHAG30
366	HOFAD96R	NADH dehydrogenase subunit 4L [Felis catus] >sp P48931 NULM_FELCA NADH-UBIQUINONE OXIDOREDUCTASE CHAIN 4L (EC 1.6.5.3). Length = 98	gi 1098532	2	253	50	52	HOFAD96
367	H2CBX07R	Nin 221 (pept unknown;221) [Bacteriophage lambda] >pir[G43011]Q1BP1L multiple specificity phosphoprotein phosphatase (EC 3.1.3.-) - phage lambda >sp P03772 PP_LAMBD SERINE/THREONINE PROTEIN PHOSPHATASE (EC 3.1.3.16). Length = 221	gi 215160	2	184	100	100	H2CBX07

368	HDPLN02R	nuclear corepressor KAP-1 [Homo sapiens] Length = 835	gi 1699027	149	454	90	90	HDPLN02
369	HT4FU27R	nuclear corepressor KAP-1 [Homo sapiens] Length = 835	gi 1699027	96	287	95	95	HT4FU27
370	HAEA126R	open reading frame A; putative [Homo sapiens] Length = 84	gi 190369	109	291	78	80	HAEA126
371	HCDAR56R	p23 [Homo sapiens] >pir A56211 A56211 progesterone receptor-related protein p23 - human >sp Q15185 Q15185 (P23). Length = 160	gi 438652	2	208	90	92	HCDAR56
372	HCDCW35R	precursor [Homo sapiens] Length = 631	gi 36049	3	155	78	84	HCDCW35
373	H2CBN76R	proteasome subunit C5 [Homo sapiens] >gnl PID e1334433 (AL031259) C5 (proteasome subunit HCS) [Homo sapiens] >pir S15973 SNHUC5 multicatalytic endopeptidase complex (EC 3.4.99.46) chain C5 - human >sp P20618 PRC5_HUMAN PROTEASOME COMPONENT C5 (EC 3.4.99.4	gnl PID d100 1116	3	464	99	99	H2CBN76
374	HAGFX49R	proteasome subunit C5 [Homo sapiens] >gnl PID e1334433 (AL031259) C5 (proteasome subunit HCS) [Homo sapiens] >pir S15973 SNHUC5 multicatalytic endopeptidase complex (EC 3.4.99.46) chain C5 - human >sp P20618 PRC5_HUMAN PROTEASOME COMPONENT C5 (EC 3.4.99.4	gnl PID d100 1116	1	288	98	100	HAGFX49

375	HNEEG64R	put. major coat protein (AA 1-341) [Bacteriophage phi-80] >pir S03314 VHBP80 major capsid protein - phage phi-80 >sp P05481 HEAD_BPPH8 MAJOR HEAD PROTEIN (GPE) (GP5) (MAJOR COAT PROTEIN). Length = 341	gi 15769	17	232	81	97	HNEEG64
376	HTXKR32R	putative nucleotide-binding protein [Homo sapiens] >pir JC4010 JC4010 nucleotide-binding protein - human >sp P53384 NBP_HUMAN NUCLEOTIDE-BINDING PROTEIN (NBP). Length = 320	gi 515644	3	374	100	100	HTXKR32
377	HAIBZ58R	putative start codon [Homo sapiens] Length = 210	gi 895845	2	433	65	65	HAIBZ58
378	H6EAF46R	rex (exclusion; 279) [Bacteriophage lambda] >gi 15068 reading frame (rex) protein [Bacteriophage 434] >pir E43010 MBPAL_rxA protein - phage lambda Length = 279	gi 215146	43	333	92	93	H6EAF46
379	H2LAW60R	ribosomal protein L27a [Homo sapiens] >pir S55914 S55914 ribosomal protein L27a - human Length = 148	gi 550017	3	545	88	88	H2LAW60
380	H2LAK40R	ribosomal protein L31 [Sus scrofa] >gi 36130 ribosomal protein L31 (AA 1-125) [Homo sapiens] >gi 57115 ribosomal protein L31 (AA 1-125) [Rattus norvegicus] >pir S05576 RSHU31 ribosomal protein L31 - human >pir A26417 RSRT31 ribosomal protein L31 - rat >gn	gn PID e276436	76	483	77	80	H2LAK40



381	H2LAY71R	ribosomal protein L35 [Homo sapiens] >pir G01477 G01477 ribosomal protein L35 - human Length = 123	gi 562074	70	495	100	100	H2LAY71
382	HCHAH62R	ribosomal protein L8 [Homo sapiens] >gi 57704 ribosomal protein L8 [Rattus rattus] >gi 1527178 ribosomal protein L8 [Mus musculus] >pir J0177 RSR L8 ribosomal protein L8, cytosolic - rat >pir JN0923 JN0923 ribosomal protein L8, cytosolic - human >gi 3851	gi 433899	1	222	76	76	HCHAH62
383	H6EEF31R	ribosomal protein S2 [Rattus norvegicus] >sp O55211 O55211 RIBOSOMAL PROTEIN S2. Length = 257	gi 2920825	1	300	89	91	H6EEF31
384	HDPBT55R	RNAse L inhibitor [Mus musculus] >sp O88793 O88793 RNAse L INHIBITOR. Length = 599	gi 3273417	71	127	81	86	HDPBT55
385	HASAW80R	S.macroura Wilms tumour protein [Sminthopsis macroura] Length = 239	gi 987118	1	162	90	98	HASAW80
386	HCHAF25R	SSR alpha subunit [Homo sapiens] >pir J38246 J38246 SSR alpha subunit - human Length = 286	gi 551638	2	421	95	95	HCHAF25
387	HLTHH84R	UMP synthase [Homo sapiens] >pir A30148 A30148 UMP synthase - human Length = 480	gi 340168	2	391	99	99	HLTHH84
388	H2CBU20R			39-	143			H2CBU20
389	HADAA62R			3	218			HADAA62
390	HADDC09R			16	174			HADDC09
391	HAIAB75R			2	211			HAIAB75

392	HAMGA37R	3	119	HAMGA37
393	HAQA110R	1	81	HAQA110
394	HBFBME95R	3	218	HBFBME95
395	HBGBH24R	1	81	HBGBH24
396	HBGBT78R	1	69	HBGBT78
397	HBGCB06R	3	140	HBGCB06
398	HBGDO01R	1	156	HBGDO01
399	HBIBJ73R	3	341	HBIBJ73
400	HBJLE85R	3	398	HBJLE85
401	HBNAD53R	2	187	HBNAD53
402	HBNAT63R	54	173	HBNAT63
403	HCE4H65R	2	193	HCE4H65
404	HCFLJ44R	92	274	HCFLJ44
405	HCHMW05R	3	221	HCHMW05
406	HCHNR50R	2	103	HCHNR50
407	HE8DS01R	2	64	HE8DS01
408	HFEBP31R	109	276	HFEBP31

409	HLDXE36R	6	167	HLDXE36
410	HLTGV28R	181	414	HLTGV28
411	HODFW25R	42	308	HODFW25
412	HOEMQ91R	1	129	HOEMQ91
413	HOGBG56R	57	386	HOGBG56
414	HOSMT44R	2	151	HOSMT44
415	HRAEE04R	51	191	HRAEE04
416	HULFN65R	3	272	HULFN65
417	HWLVW23R	1	153	HWLVW23
418	HWLWE77R	149	289	HWLWE77

The first column of Table 1 shows the "SEQ ID NO:" for each of the 418 breast/ovarian cancer antigen polynucleotide sequences of the invention.

The second column in Table 1, provides a unique "Sequence/Contig ID" identification for each breast, ovarian, breast cancer and/or ovarian cancer associated sequence. The third column in Table 1, "Gene Name," provides a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database, such as GenBank (NCBI). The great majority of the cDNA sequences reported in Table 1 are unrelated to any sequences previously described in the literature. The fourth column, in Table 1, "Overlap," provides the database accession no. for the database sequence having similarity. The fifth and sixth columns in Table 1 provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. In one embodiment, the invention provides a protein comprising, or alternatively consisting of, a polypeptide encoded by the portion of SEQ ID NO:X delineated by the nucleotide position nos. "Start" and "End". Also provided are polynucleotides encoding such proteins and the complementary strand thereto. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity) observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence.

The ninth column of Table 1 provides a unique "Clone ID" for a clone related to each contig sequence. This clone ID references the cDNA clone which contains at least the 5' most sequence of the assembled contig and at least a portion of SEQ ID NO:X was determined by directly sequencing the referenced clone. The reference clone may have more sequence than described in the sequence listing or the clone may have less. In the vast majority of cases, however, the clone is believed to encode a full-length polypeptide. In the case where a clone is not full-length, a full-length cDNA can be obtained by methods described elsewhere herein.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, or more of any one or more of these public ESTs are optionally excluded from the invention.

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing as SEQ ID NO:1 through SEQ ID NO:418) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing as SEQ

ID NO:418 through SEQ ID NO:836) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X has uses including, but not limited to, in designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the related cDNA clone  
5 contained in a library deposited with the ATCC. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling immediate applications in chromosome mapping, linkage analysis, tissue identification and/or typing, and a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y have uses that include, but are not limited to, generating antibodies which  
10 bind specifically to the breast/ovarian cancer antigen polypeptides, or fragments thereof, and/or to the breast/ovarian cancer antigen polypeptides encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions  
15 of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over  
20 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing the related cDNA clone  
25 (deposited with the ATCC, as set forth in Table 1). The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. Further, techniques known in the art can be used to verify the nucleotide sequences of SEQ ID NO:X.

The predicted amino acid sequence can then be verified from such deposits.  
30 Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to vectors or plasmids which include such DNA sequences, as well as the use of the DNA sequences. The material deposited with the ATCC on:

5    **Table 2**

ATCC Deposits	Deposit Date	ATCC Designation Number
LP01, LP02, LP03, LP04, LP05, LP06, LP07, LP08, LP09, LP10, LP11,	May-20-97	209059, 209060, 209061, 209062, 209063, 209064, 209065, 209066, 209067, 209068, 209069
LP12	Jan-12-98	209579
LP13	Jan-12-98	209578
LP14	Jul-16-98	203067
LP15	Jul-16-98	203068
LP16	Feb-1-99	203609
LP17	Feb-1-99	203610
LP20	Nov-17-98	203485
LP21	Jun-18-99	PTA-252
LP22	Jun-18-99	PTA-253
LP23	Dec-22-99	PTA-1081

each is a mixture of cDNA clones derived from a variety of human tissue and cloned in either a plasmid vector or a phage vector, as shown in Table 5. These deposits are referred to as  
10    "the deposits" herein. The tissues from which the clones were derived are listed in Table 5, and the vector in which the cDNA is contained is also indicated in Table 5. The deposited material includes the cDNA clones which were partially sequenced and are related to the SEQ ID NO:X described in Table 1 (column 9). Thus, a clone which is isolatable from the ATCC Deposits by use of a sequence listed as SEQ ID NO:X may include the entire coding  
15    region of a human gene or in other cases such clone may include a substantial portion of the coding region of a human gene. Although the sequence listing lists only a portion of the DNA sequence in a clone included in the ATCC Deposits, it is well within the ability of one skilled in the art to complete the sequence of the DNA included in a clone isolatable from the

ATCC Deposits by use of a sequence (or portion thereof) listed in Table 1 by procedures hereinafter further described, and others apparent to those skilled in the art.

Also provided in Table 5 is the name of the vector which contains the cDNA clone. Each vector is routinely used in the art. The following additional information is provided for  
5 convenience.

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res.* 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res.* 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies* 5:58-61 (1992)) are commercially available from Stratagene  
10 Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain  
15 XL-1 Blue, also available from Stratagene.

Vectors pSport1, pCMVSPORT 1.0, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus*  
20 15:59 (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res.*  
25 16:9677-9686 (1988) and Mead, D. et al., *Bio/Technology* 9: (1991).

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in a deposited cDNA clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include, but are not limited to, preparing probes or primers from the  
30 disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in the related cDNA clone in the deposit, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or the related cDNA clone (See, e.g., columns 1 and 9 of Table 1). The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the the dDNA in the related cDNA clone contained in a deposited library, are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of, the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the complement of the coding strand of the related cDNA clone contained in a deposited library.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would unduly burden the disclosure of this application. Accordingly, for each "Contig Id" listed in the first column of Table 3, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described in the second column of Table 3 by the general formula of a-b, each of which are uniquely defined for the SEQ ID NO:X corresponding to that Contig Id in Table 1. Additionally, specific embodiments are directed to polynucleotide sequences excluding at least one, two, three, four, five, ten, or more of the specific polynucleotide sequences referenced by the Genbank Accession No. for each Contig Id which may be



included in column 3 of Table 3. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example.

Table 3

Sequence/ Contig ID	General formula	Genbank Accession No.
419266	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1899 of SEQ ID NO:1, b is an integer of 15 to 1913, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where b is greater than or equal to a + 14.	T68585, T68665, T86313, T86314, R12356, R31374, R32873, R37282, R84617, R85369, R99171, H48474, N23871, N58201, N74557, W90334, AA031318, AA031427, AA130231, AA256587
429114	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1411 of SEQ ID NO:2, b is an integer of 15 to 1425, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:2, and where b is greater than or equal to a + 14.	R20542, R42676, R42676, R20542, R61501, H08662, H77556, H97365, N24198, N33135, N74546, N93573, W02941, W52194, AA004624, AA004721, AA046710, AA235395, AA235479
506777	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 340 of SEQ ID NO:3, b is an integer of 15 to 354, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:3, and where b is greater than or equal to a + 14.	
508678	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 500 of SEQ ID NO:4, b is an integer of 15 to 514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:4, and where b is greater than or equal to a + 14.	W37175, AA121532, AA127694
508968	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	T71941, T94428, T94514, H02313, N26913, N47870, N66244, N92418, W31301, W42459, W42564, AA084031, AA126786, AA258050, AA459772

	formula of a-b, where a is any integer between 1 to 2021 of SEQ ID NO:5, b is an integer of 15 to 2035, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:5, and where b is greater than or equal to a + 14.	
509029	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1182 of SEQ ID NO:6, b is an integer of 15 to 1196, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:6, and where b is greater than or equal to a + 14.	R11213, R11271, H14072, H14071, H51531, H66637, H66636, W23707, W35307, AA025586, AA025710, AA058796, AA113917
519726	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 610 of SEQ ID NO:7, b is an integer of 15 to 624, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:7, and where b is greater than or equal to a + 14.	AA236015, AA236085, AA256106
522632	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 287 of SEQ ID NO:8, b is an integer of 15 to 301, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:8, and where b is greater than or equal to a + 14.	
524655	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 672 of SEQ ID NO:9, b is an integer of 15 to 686, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:9, and where b is greater than or equal to a + 14.	T66495, R15869, R39696, H16266, H20784, H22599, N68150, W58001, W57856
525847	Preferably excluded from the present	

	invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 383 of SEQ ID NO:10, b is an integer of 15 to 397, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:10, and where b is greater than or equal to a + 14.	
530306	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 549 of SEQ ID NO:11, b is an integer of 15 to 563, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.	
532818	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 429 of SEQ ID NO:12, b is an integer of 15 to 443, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.	AA188990, AA191040
533385	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2424 of SEQ ID NO:13, b is an integer of 15 to 2438, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.	
533532	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2333 of SEQ ID NO:14, b is an integer of 15 to 2347, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	T94240, T77619, R13236, R17515, R33142, R33294, R39249, R40318, R42609, R42609, R40318, R75952, H03594, H12337, H12391, H70913, H70916, H70996, H71001, H87858, H70913, N21374, N31326, N35068, N35435, N43807, N45045, W46431, W46486, W51917, AA019546, AA018858, AA056764, AA056767, AA058441, AA058445, AA083228, AA083269, AA115939, AA122236, AA147307, AA159802,

	NO:14, and where b is greater than or equal to a + 14.	AA165015, AA165642, AA181869, AA186834, AA252269, AA255892, AA463239, AA463240
534852	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1992 of SEQ ID NO:15, b is an integer of 15 to 2006, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.	T55469, T63434, R10603, R10604, H50597, H92640, H94634, W39162, W93243, W94634, W94719, N90240, AA053667, AA167312, AA253414, AA253389
537910	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 972 of SEQ ID NO:16, b is an integer of 15 to 986, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.	R23785
538460	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1575 of SEQ ID NO:17, b is an integer of 15 to 1589, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.	R13084, R40514, R40514, R55303, R55402, W67446
539577	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 832 of SEQ ID NO:18, b is an integer of 15 to 846, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.	T49208, N35488, AA088419, AA127572, AA127649, AA156316, AA169250
548379	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2178 of SEQ ID NO:19, b	R23778, H70824

	is an integer of 15 to 2192, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.	
548489	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 997 of SEQ ID NO:20, b is an integer of 15 to 1011, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.	T49861, T49862, T56225, T56367, T72170, T72948, T92867, T74728, R08625, R08719, R17408, R24674, R25174, R25378, R25997, R26800, R28401, R31330, R31589, R42642, R45259, R42642, R45259, R62552, R62553, R66386, R67726, R68781, R68878, H25120, H25121, H41115, H41190, H41191, R84227, R87629, H53386, H64419, H64476, H72640, H72641, H64419, H99301, N22341, N25846, N29370, N29843, N47918, N57261, N59763, N63813, N94171, W23786, W45524, W72111, W77797, AA010718, AA011164, AA033553, AA033554, AA062727, AA062741, AA062784, AA069811, AA075470, AA075471, AA081844, AA083492, AA084442, AA100358, AA126263, AA126354, AA136544, AA136648, AA146862, AA146863, AA179509, AA179540, AA179775, AA180492, AA181719, AA188903, AA189140, AA226959, AA227247
548595	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2005 of SEQ ID NO:21, b is an integer of 15 to 2019, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.	T61537, T69836, R10679, R42501, R46798, R42501, R46798, H05289, H05822, H12239, H16816, H40312, R86905, R86985, N21432, N73268, W73102, N91565, AA033533, AA053026, AA121547, AA127684, AA190356, AA195451, AA226965, AA232522, AA258142
549337	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2008 of SEQ ID NO:22, b is an integer of 15 to 2022, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.	
549777	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1112 of SEQ ID NO:23, b	T81557, R27931, R38730, R39493, R39494, R66845, R67942, R69099, R69214, R69613, R69703, R69740, R72430, R72478, R73090, R73091, R73872, R73955, R82662, R82715, H01096, H01097, H72113, N76139, W58493, W72884, W74409, W94644, W92532,

	is an integer of 15 to 1126, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.	AA022916, AA022917, AA039661, AA039660, AA043439, AA054965, AA152376, AA148360, AA181225, AA188435
553091	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2584 of SEQ ID NO:24, b is an integer of 15 to 2598, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.	
553827	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 397 of SEQ ID NO:25, b is an integer of 15 to 411, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.	
556350	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 643 of SEQ ID NO:26, b is an integer of 15 to 657, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.	T70920, R01856, R37402, H21077, H21531, R94734, N29364, N32255, N80553, W07675, W58340, W58661, W67208, W67352, AA039658, AA039659, AA046392, AA055650, AA058365, AA070442, AA088882, AA102056, AA134144, AA165363, AA171617, AA173761, AA173771, AA252260, AA464575, AA464679
556351	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1889 of SEQ ID NO:27, b is an integer of 15 to 1903, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.	T70981, R01855, R13494, H21076, H24431, H24460, R94817, N47912, AA040086, AA040133, AA055706, AA056162, AA058484, AA102055, AA102304, AA130304, AA173608, AA195879
557007	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	H13846, H13894, H16354, H20742, H20743, R97935, R97936, H87445, N29633, AA015991, AA045671, AA045670, AA099154, AA099252

	sequence described by the general formula of a-b, where a is any integer between 1 to 1319 of SEQ ID NO:28, b is an integer of 15 to 1333, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.	
558140	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1313 of SEQ ID NO:29, b is an integer of 15 to 1327, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.	T62991, W58535, W58500, AA053629, AA083878, AA112892, AA157250, AA157345, AA194089, AA253436, AA250750
558456	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 695 of SEQ ID NO:30, b is an integer of 15 to 709, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.	
558708	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1094 of SEQ ID NO:31, b is an integer of 15 to 1108, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.	R38385, W24640, W48793, W49619
574789	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 512 of SEQ ID NO:32, b is an integer of 15 to 526, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.	N49156



578203	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 541 of SEQ ID NO:33, b is an integer of 15 to 555, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.	AA149853
585385	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 333 of SEQ ID NO:34, b is an integer of 15 to 347, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.	
588869	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 736 of SEQ ID NO:35, b is an integer of 15 to 750, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.	
597076	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1277 of SEQ ID NO:36, b is an integer of 15 to 1291, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.	
598656	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1521 of SEQ ID NO:37, b is an integer of 15 to 1535, where both a and b correspond to the positions of	

	nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.	
611880	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 281 of SEQ ID NO:38, b is an integer of 15 to 295, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.	
614329	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1286 of SEQ ID NO:39, b is an integer of 15 to 1300, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.	T49777, T51334, T49778, T66835, T66836, T78401, R33579, R33684, R34361, R34476, R72556, R75702, H01591, H02719, H13232, H13599, H13942, H13943, H63376, H80729, H80730, H89353, H89539, H99395, N26995, N32930, N40116, N42081, N50408, N50460, N63978, N67308, N92847, W46413, AA126994, AA128141, AA146958, AA146957, AA425764
616066	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 201 of SEQ ID NO:40, b is an integer of 15 to 215, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.	
620956	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 460 of SEQ ID NO:41, b is an integer of 15 to 474, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.	
621889	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	

	between 1 to 411 of SEQ ID NO:42, b is an integer of 15 to 425, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.	
624017	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1173 of SEQ ID NO:43, b is an integer of 15 to 1187, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.	T61010, AA071044, AA088260, AA098798, AA102017, AA100707, AA111883, AA113305, AA121495, AA133235, AA131438, AA132011, AA132866, AA143457, AA146581, AA146805, AA146928, AA155613, AA155609, AA158090, AA158263, AA164694, AA165591, AA176429, AA226820
651784	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 501 of SEQ ID NO:44, b is an integer of 15 to 515, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.	W32583, W68240, W94174, AA251670, AA252011, AA252266, AA425209
651826	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1485 of SEQ ID NO:45, b is an integer of 15 to 1499, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.	T47384, T47385, T60137, T60194, T71947, T95050, T95146, R25340, R25476, R26117, R26301, R27566, R27664, R28180, R33393, R35872, R35873, R36483, R48329, R48438, R62139, R62244, R66007, R66008, R66764, R70718, R70719, R73674, R73761, R74132, R76569, R76643, R77265, R77312, R78827, R79686, R79687, R81316, R81751, H00804, H00891, H01415, H01416, H02522, H03673, H13925, H13926, H24743, H26369, H26727, H26728, H27132, H27480, H27663, H28192, H28235, H41929, H41977, H42604, H43209, H43258, H45278, H45348, H53585, H53906, H61785, H61786, H78337, H78338, H87337, H87871, H95183, N27090, N27092, N40499, N40502, N99158, W24165, W60193, AA039817, AA041344, AA074512, AA079058, AA079156, AA079157, AA085829, AA085974, AA100095, AA113304, AA142843, AA149898, AA156331, AA157820, AA157895, AA158552, AA159177, AA176093, AA179607, AA179608, AA176333, AA187637, AA186769, AA188622, AA188742, AA188975
653282	Preferably excluded from the present	

	invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 379 of SEQ ID NO:46, b is an integer of 15 to 393, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.	
657122	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 224 of SEQ ID NO:47, b is an integer of 15 to 238, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.	
661442	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 925 of SEQ ID NO:48, b is an integer of 15 to 939, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.	R18101, AA424721
664914	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1757 of SEQ ID NO:49, b is an integer of 15 to 1771, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.	T86944, T87027, R11421, T81153, T81380, R17243, R17453, R19171, R27826, R27927, R35295, R35940, R41854, R42800, R48191, R48192, R49457, R51209, R52247, R53413, R41854, R42800, R49457, R55257, R55475, R59472, R71390, R81811, R81915, H05137, H07974, H30702, H42552, H57923, H58015, N71127, N74282, N75329, N93224, W01557, W04382, W04780, W23438, W35253, W38865, AA176204, AA194869, AA199875, AA251414
666654	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 383 of SEQ ID NO:50, b is an integer of 15 to 397, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	

	NO:50, and where b is greater than or equal to a + 14.	
667084	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1621 of SEQ ID NO:51, b is an integer of 15 to 1635, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.	R71869, R71870, H22387, H27160, H46592, H61204, H62108, N25274, N94410, AA026642, AA069188, AA069189, AA076423, AA076388, AA076533, AA076540, AA122346, AA121039, AA121092, AA133121, AA143471, AA143470, AA143728, AA156363, AA156404, AA158498, AA159190, AA159201, AA159286, AA160335, AA159837, AA159573, AA160367, AA159548, AA160456, AA160697, AA160789, AA179329, AA181540, AA182669, AA186881, AA186887, AA188535, AA188540, AA190669, AA190973, AA191557, AA235457, AA458511, AA418203
667380	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1766 of SEQ ID NO:52, b is an integer of 15 to 1780, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.	T87574, R10276, R10277, T79847, R49790, R49832, R59538, R59539, R86940, R87067, R87722, R98577, R98578, R99022, R99795, H72692, H93036, H93942, H93941, N54059, N62326, N64719, N66726, N73888, N74171, N91734, N93505, W02054, W03949, W04337, W21317, AA192562, AA192563, AA223984, AA224049
669530	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 476 of SEQ ID NO:53, b is an integer of 15 to 490, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.	T49160, T49161, H41659, R88196, W60799, W60930, AA046915, AA046972, AA069703, AA464334
671315	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1930 of SEQ ID NO:54, b is an integer of 15 to 1944, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.	
671993	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	

	between 1 to 980 of SEQ ID NO:55, b is an integer of 15 to 994, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.	
674618	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 314 of SEQ ID NO:56, b is an integer of 15 to 328, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a + 14.	
675027	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1475 of SEQ ID NO:57, b is an integer of 15 to 1489, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.	T86474, AA133454, AA203346
677202	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1269 of SEQ ID NO:58, b is an integer of 15 to 1283, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.	T47486, T47487, T47666, T50413, T50493, T50519, T51852, T53234, T57067, T60776, T40856, T93579, T94432, T94435, T96391, R43542, R43542, H21618, H73240, H88867, H88868, H89122, H88868, H89122, N21997, N22243, N22815, N45720, N48998, N52063, N59239, N62103, N66419, N66708, N66782, N67139, N67283, N67447, N68047, N70159, N71198, N74676, N76707, N78333, N80016, N92971, N93518, W05738, W45694, W48845, W80602, AA057801, AA063330, AA064827, AA065165, AA065178, AA065179, AA069552, AA070491, AA070949, AA070969, AA071333, AA071358, AA074331, AA081280, AA111928, AA112051, AA132018, AA132121, AA147357, AA157065, AA157085, AA157890, AA160054, AA181729, AA182765, AA187698, AA186444, AA196168, AA196244, AA224187
678504	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 726 of SEQ ID NO:59, b is	

	an integer of 15 to 740, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.	
678985	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1277 of SEQ ID NO:60, b is an integer of 15 to 1291, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.	
682161	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 957 of SEQ ID NO:61, b is an integer of 15 to 971, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.	
683476	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 604 of SEQ ID NO:62, b is an integer of 15 to 618, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.	
691146	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1124 of SEQ ID NO:63, b is an integer of 15 to 1138, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where b is greater than or equal to a + 14.	T48865, T48866, T48901, T47562, T48902, T54258, T54365, T69783, T70768, R08012, R09058, R09059, T83437, T84082, T99021, R09059, R19174, R21551, R22562, R28286, R48757, R48758, R49683, R49683, R62406, R62407, R70222, R75607, R77000, R78400, R78401, R80802, H02840, H03734, H24549, H26291, H26447, H27912, H43630, H47817, R83903, R83904, R94147, H49533, H49773, H50716, H50820, H87446, H87553, H93471, H93472, H98814, N22867, N32137, N32762, N34334, N35009, N36932, N43763, N46205, N52251, N56805, N72290, N95794, W02713, W02886, W17176, W24905, W25571, W25688.

		W67795, W72687, W72962, W77793, W79704, W81376, W86301, W86316, AA025519, AA025959, AA026653, AA029556, AA029704, AA079472, AA121306, AA136679, AA148681, AA148680, AA181745, AA425923
693589	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 404 of SEQ ID NO:64, b is an integer of 15 to 418, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:64, and where b is greater than or equal to a + 14.	
694991	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2822 of SEQ ID NO:65, b is an integer of 15 to 2836, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:65, and where b is greater than or equal to a + 14.	
698303	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2291 of SEQ ID NO:66, b is an integer of 15 to 2305, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:66, and where b is greater than or equal to a + 14.	T83582, T84417, T85606, R66380, R67111, R76298, H96019, H96020, N25659, N25661, N34260, N34263, N70618, W05500, W15421, W23670, W39659, AA015855, AA033569, AA033570, AA044566, AA044583, AA178933, AA179025
698669	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1893 of SEQ ID NO:67, b is an integer of 15 to 1907, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where b is greater than or equal to a + 14.	T47115, T47116, R48786, R48893, R55495, R71847, R78934, R79033, R82776, H26587, H27077, R97760, H59232, H79115, H79116, N22948, N23658, N26858, N28757, N39967, N71599, W24648, W60157, W67490, W67491, W67815, W72921, W94215, AA009634, AA026899, AA026900, AA029244, AA029040, AA031846, AA031847, AA032073, AA034285, AA034992, AA036865, AA037006, AA040908, AA039990, AA040521, AA040522, AA040773, AA043726, AA044071, AA044182, AA042948, AA043067, AA046606, AA046721, AA062914, AA074334, AA076039, AA076203, AA079763, AA079764, AA082550, AA085926, AA099318,



		AA099836, AA102385, AA101039, AA101040, AA112571, AA112572, AA114828, AA114951, AA128001, AA128082, AA126986, AA128134, AA128459, AA129910, AA131403, AA131503, AA147437, AA147438, AA150961, AA151051, AA156785, AA156855, AA157912, AA157913, AA158544, AA158545, AA158554, AA158553, AA211822, AA460840, AA461144
705696	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 801 of SEQ ID NO:68, b is an integer of 15 to 815, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where b is greater than or equal to a + 14.	H20141, H20156, H20236, H20250, H49965, H50007, H50487, W92252, AA045116, AA134141, AA142968
706393	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1136 of SEQ ID NO:69, b is an integer of 15 to 1150, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where b is greater than or equal to a + 14.	T48975, T51242, T51357, T59673, T59807, T62725, T62875, T72330, T97577, R01168, R21893, R22365, R35745, R41863, R41863, R63676, R65881, R72862, R73334, R75659, R75767, H02871, H03430, H03512, H14924, H23660, H30020, H30277, H39675, H40069, H40278, H40526, H41667, H41700, H43170, H43670, H45130, H45172, H45173, H45433, H46542, H46952, H46953, H62390, H78695, H78777, H84781, H85405, H92309, N20534, N33402, N38945, N57790, N57945, N59752, W94488, W94489, AA044423, AA043057, AA081370, AA081371, AA099447, AA112623, AA112622, AA143199, AA143214, AA149467, AA149553, AA157049, AA157201, AA157952, AA157953, AA158049, AA158435, AA158837, AA158841, AA161074, AA161078, AA180395, AA251447, AA419021, AA428783, AA429093
707357	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 330 of SEQ ID NO:70, b is an integer of 15 to 344, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where b is greater than or equal to a + 14.	
707360	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	

	formula of a-b, where a is any integer between 1 to 434 of SEQ ID NO:71, b is an integer of 15 to 448, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where b is greater than or equal to a + 14.	
707375	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2811 of SEQ ID NO:72, b is an integer of 15 to 2825, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where b is greater than or equal to a + 14.	T54138, T65139, T65330, T80324, T83140, R00512, R00612, R19513, R31469, R31470, R47795, R77921, R78022, R80012, H02327, H02429, H06404, H06405, H08607, H08608, H14264, H18370, H19266, H19267, H21399, H21471, H47094, H47185, R85467, R87496, R87501, R87581, R88189, R88226, R88227, N23376, N32357, N58463, N66212, N93661, N99103, W19083, W24383, W68601, W68602, W68723, W68745, AA016149, AA040296, AA056973, AA135439, AA135519, AA135580, AA135856, AA158858, AA161122, AA226730, AA226764, AA227471, AA227481, AA232259
707754	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 496 of SEQ ID NO:73, b is an integer of 15 to 510, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:73, and where b is greater than or equal to a + 14.	
711172	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 444 of SEQ ID NO:74, b is an integer of 15 to 458, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:74, and where b is greater than or equal to a + 14.	
712248	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 363 of SEQ ID NO:75, b is an integer of 15 to 377, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:75, and where b is greater than or	

	equal to $a + 14$ .	
715445	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2056 of SEQ ID NO:76, b is an integer of 15 to 2070, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:76, and where b is greater than or equal to $a + 14$ .	T88778, T97557, T97604, R17189, R27615, R30849, R41740, R48616, R41740, H12351, R93768, R98882, R98972, H59983, N23156, N32736, N34539, N55086, N62785, N67224, N77297, N78823, N79734, W07252, W90651, AA037793, AA037794, AA055196, AA055286, AA113425, AA233917, AA234165, AA258602, AA258548, AA426581, AA429080
716362	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 983 of SEQ ID NO:77, b is an integer of 15 to 997, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:77, and where b is greater than or equal to $a + 14$ .	
716835	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1319 of SEQ ID NO:78, b is an integer of 15 to 1333, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:78, and where b is greater than or equal to $a + 14$ .	
716947	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 546 of SEQ ID NO:79, b is an integer of 15 to 560, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:79, and where b is greater than or equal to $a + 14$ .	
717685	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3189 of SEQ ID NO:80, b is an integer of 15 to 3203, where both a	T54040, N35800, W45088, AA122232, AA121109, AA126030, AA126152, AA155618, AA155656

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:80, and where b is greater than or equal to $a + 14$ .	
719755	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1696 of SEQ ID NO:81, b is an integer of 15 to 1710, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:81, and where b is greater than or equal to $a + 14$ .	
720389	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1365 of SEQ ID NO:82, b is an integer of 15 to 1379, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:82, and where b is greater than or equal to $a + 14$ .	
720903	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 664 of SEQ ID NO:83, b is an integer of 15 to 678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:83, and where b is greater than or equal to $a + 14$ .	
721348	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2789 of SEQ ID NO:84, b is an integer of 15 to 2803, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:84, and where b is greater than or equal to $a + 14$ .	
721562	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	

	formula of a-b, where a is any integer between 1 to 1264 of SEQ ID NO:85, b is an integer of 15 to 1278, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:85, and where b is greater than or equal to a + 14.	
722775	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2571 of SEQ ID NO:86, b is an integer of 15 to 2585, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:86, and where b is greater than or equal to a + 14.	
724463	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 371 of SEQ ID NO:87, b is an integer of 15 to 385, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:87, and where b is greater than or equal to a + 14.	
727501	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2486 of SEQ ID NO:88, b is an integer of 15 to 2500, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:88, and where b is greater than or equal to a + 14.	
728418	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1395 of SEQ ID NO:89, b is an integer of 15 to 1409, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:89, and where b is greater than or equal to a + 14.	
728920	Preferably excluded from the present	

	invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1322 of SEQ ID NO:90, b is an integer of 15 to 1336, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:90, and where b is greater than or equal to a + 14.	
732958	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 773 of SEQ ID NO:91, b is an integer of 15 to 787, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:91, and where b is greater than or equal to a + 14.	
733134	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1643 of SEQ ID NO:92, b is an integer of 15 to 1657, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:92, and where b is greater than or equal to a + 14.	T49547, T49558, T49559, T49560, T49561, T49649, T49650, T70062, T70129, T75532, T95137, R17573, T27052, R19790, R42912, R52618, R53272, R42912, R59922, R59923, R65930, H08841, H08925, H47546, H47547, H47774, H47784, H48119, H64949, H64950, H69959, H69960, H80517, H80569, H81281, H81337, H87618, H87619, H88959, H89042, H95657, H95712, H95729, H88959, H98860, N20108, N23582, N27446, N34733, N49675, N51841, N75517, N78965, N93975, W05310, W17334, W40344, W52084, W52929, W72818, W72819, W86046, W92307, W92294, AA009783, AA009892, AA022930, AA022980, AA024699, AA024734, AA037408, AA045887, AA045888, AA062821, AA081026, AA082088, AA082420, AA102801, AA199861, AA199931, AA220961, AA223217, AA223456, AA224153, AA224177, AA224137, AA224138, AA224341, AA232349, AA232533, AA232117, AA458900, AA459095, AA463299
734099	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 471 of SEQ ID NO:93, b is an integer of 15 to 485, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:93, and where b is greater than or	R22895, H87448

	equal to $a + 14$ .	
734599	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 750 of SEQ ID NO:94, b is an integer of 15 to 764, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:94, and where b is greater than or equal to $a + 14$ .	
736019	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 693 of SEQ ID NO:95, b is an integer of 15 to 707, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:95, and where b is greater than or equal to $a + 14$ .	T41219, T50359, T56829, T58426, T58458, T60928, T60984, T64158, T64287, R27157, H03484, H03579, H22546, H22547, H28310, H44067, H44146, R83796, H48481, H48645, H57243, H66162, H66163, H82370, N21110, N21188, N27461, N29155, N29743, N31124, N32398, N39884, N56818, N57165, N57228, N57403, N68904, N73978, N77833, N93027, N93818, N67112, W00894, W00923, W02234, W16676, W21379, W44969, AA064843, AA070697, AA070876, AA071332, AA071265, AA076379, AA076308, AA079524, AA079572, AA081231, AA081401, AA083774, AA083775, AA130308, AA130309, AA132056, AA132160, AA143132, AA146882, AA146883, AA165057, AA164722, AA166939, AA181133, AA187371, AA187804, AA188118, AA186447, AA186448, AA187105, AA187150, AA188273
738268	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 801 of SEQ ID NO:96, b is an integer of 15 to 815, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:96, and where b is greater than or equal to $a + 14$ .	T48287, T48288, T54477, T54511, R34064, R36907, R49496, R49496, R75625, R75724, H12225, H16384, H19466, H19543, H42166, H42988, H54780, H99297, N22733, N26471, N74933, N93468, W15461, W47542, W47590, N90997, AA010700, AA010701, AA056728, AA088699, AA126219, AA132934, AA156291, AA165516, AA165558, AA176293, AA173448, AA189056, AA233515, AA459831, AA460011
738911	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 644 of SEQ ID NO:97, b is an integer of 15 to 658, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:97, and where b is greater than or equal to $a + 14$ .	H22593, H52836

739226	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 235 of SEQ ID NO:98, b is an integer of 15 to 249, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:98, and where b is greater than or equal to a + 14.	T57824, N63155, AA027845
739527	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 738 of SEQ ID NO:99, b is an integer of 15 to 752, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:99, and where b is greater than or equal to a + 14.	
740710	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3045 of SEQ ID NO:100, b is an integer of 15 to 3059, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:100, and where b is greater than or equal to a + 14.	
742980	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1668 of SEQ ID NO:101, b is an integer of 15 to 1682, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:101, and where b is greater than or equal to a + 14.	T71993, R12901, R40053, H14591, H14696, R83485, H50584, H50585, H89958, H89966, H89973, H89980, N26005, N34777, N36638, N36637, N44503, N67682, N76121, N79613, W03491, W05571, W31276, W49653, W49727, AA009708, AA009798, AA035612, AA042894, AA043030, AA062953, AA115370, AA133278, AA181268, AA181269, AA193206
744331	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 924 of SEQ ID NO:102, b is an integer of 15 to 938, where both a and b correspond to the positions of	R25354, R49789, R71735, R71740, H73502, H79224, H87423, H99515, H99516, N24751, N32707, N44511, N52325, N67764, N75095, N93879, W40372, W69127, W69094, W74698, W74736, AA026984, AA035176, AA149088, AA262739, AA464357, AA430724



	nucleotide residues shown in SEQ ID NO:102, and where b is greater than or equal to a + 14.	
744751	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1998 of SEQ ID NO:103, b is an integer of 15 to 2012, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:103, and where b is greater than or equal to a + 14.	
745750	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1080 of SEQ ID NO:104, b is an integer of 15 to 1094, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:104, and where b is greater than or equal to a + 14.	
746285	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2283 of SEQ ID NO:105, b is an integer of 15 to 2297, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:105, and where b is greater than or equal to a + 14.	T87719, T87928, R99975, R99976, H64714, H65205, H92423, H65205, N47296, N48612, N58085, N58926, N64294, N64508, N72401, N80294, N93405, W04791, W21447, W94582, W95317, AA024856, AA024939, AA037672, AA037673, AA070416, AA075508, AA075507, AA101263, AA148029, AA147953, AA169726, AA171461, AA173095, AA464821
746416	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 428 of SEQ ID NO:106, b is an integer of 15 to 442, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:106, and where b is greater than or equal to a + 14.	
747851	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	N44767, W44754

	between 1 to 1005 of SEQ ID NO:107, b is an integer of 15 to 1019, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:107, and where b is greater than or equal to a + 14.	
750632	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 697 of SEQ ID NO:108, b is an integer of 15 to 711, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:108, and where b is greater than or equal to a + 14.	H48882, W23677, W35110, AA133857
751315	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 729 of SEQ ID NO:109, b is an integer of 15 to 743, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:109, and where b is greater than or equal to a + 14.	
754009	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 781 of SEQ ID NO:110, b is an integer of 15 to 795, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:110, and where b is greater than or equal to a + 14.	
754634	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1318 of SEQ ID NO:111, b is an integer of 15 to 1332, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:111, and where b is greater than or equal to a + 14.	N21429
756637	Preferably excluded from the present invention are one or more	N44651, W76461

	polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 729 of SEQ ID NO:112, b is an integer of 15 to 743, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:112, and where b is greater than or equal to a + 14.	
756833	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1676 of SEQ ID NO:113, b is an integer of 15 to 1690, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:113, and where b is greater than or equal to a + 14.	
756878	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 606 of SEQ ID NO:114, b is an integer of 15 to 620, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:114, and where b is greater than or equal to a + 14.	R12122
757332	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 528 of SEQ ID NO:115, b is an integer of 15 to 542, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:115, and where b is greater than or equal to a + 14.	
760835	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 511 of SEQ ID NO:116, b is an integer of 15 to 525, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:116, and where b is greater than or	

	equal to $a + 14$ .	
761760	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 714 of SEQ ID NO:117, b is an integer of 15 to 728, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:117, and where b is greater than or equal to $a + 14$ .	
762520	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 934 of SEQ ID NO:118, b is an integer of 15 to 948, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:118, and where b is greater than or equal to $a + 14$ .	T86617, T86618, R47814, R49961, R71921, R71968, H28225, H28275, R94939, R95025, R97173, R97174, R99726, R99904, H52435, H52436, H58879, H58880, H66345, H66395, H80709, H80710, W87663, W87664, AA046620, AA046867, AA055456, AA102380, AA121314, AA150579, AA197300
764461	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 197 of SEQ ID NO:119, b is an integer of 15 to 211, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:119, and where b is greater than or equal to $a + 14$ .	
764517	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1294 of SEQ ID NO:120, b is an integer of 15 to 1308, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:120, and where b is greater than or equal to $a + 14$ .	
765132	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2502 of SEQ ID NO:121, b is an integer of 15 to 2516, where both	

	a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:121, and where b is greater than or equal to a + 14.	
765667	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1125 of SEQ ID NO:122, b is an integer of 15 to 1139, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:122, and where b is greater than or equal to a + 14.	T81691, N27595
767113	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2100 of SEQ ID NO:123, b is an integer of 15 to 2114, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:123, and where b is greater than or equal to a + 14.	
767204	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 569 of SEQ ID NO:124, b is an integer of 15 to 583, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:124, and where b is greater than or equal to a + 14.	
767400	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1973 of SEQ ID NO:125, b is an integer of 15 to 1987, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:125, and where b is greater than or equal to a + 14.	
767962	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	T59753, R21255, R21256, R23274, R23364, R71913, R71956, H12633, H12686, H99087, N26954, N33518, N43798, N62998, N66835, N71124, N71156, N74144, N79907, W01554,

	formula of a-b, where a is any integer between 1 to 1437 of SEQ ID NO:126, b is an integer of 15 to 1451, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:126, and where b is greater than or equal to a + 14.	W05537, W19994, W44368, W46357, W46193, W47163, W47284, W52537, W55854, W80804, W80878, W92021, W92022, N90420, AA002178, AA022578, AA022579, AA029899, AA029987, AA034181, AA036856, AA036913, AA043237, AA043566, AA071518, AA082340, AA122159, AA120962, AA146944, AA147449, AA148081, AA151266, AA151267, AA156459
768040	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1220 of SEQ ID NO:127, b is an integer of 15 to 1234, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:127, and where b is greater than or equal to a + 14.	
769956	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 849 of SEQ ID NO:128, b is an integer of 15 to 863, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:128, and where b is greater than or equal to a + 14.	R68817, R68925, R75906, H14626, H82146, H93109, H93237, N32098, N35721, N45410, N75570, W03043, W04850, AA029607, AA262861, AA463956, AA464092
770133	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1224 of SEQ ID NO:129, b is an integer of 15 to 1238, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:129, and where b is greater than or equal to a + 14.	
770289	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 365 of SEQ ID NO:130, b is an integer of 15 to 379, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:130, and where b is greater than or equal to a + 14.	

771964	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1772 of SEQ ID NO:131, b is an integer of 15 to 1786, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:131, and where b is greater than or equal to a + 14.	T53984, T55243, T51230, T77632, T91326, T80819, T81219, T84909, T95454, T97320, T99226, T99269, R16575, R16634, R19765, R22987, R23096, R33095, R33188, R37437, R39255, R45185, R45185, R62594, R62642, H03891, H03892, H08679, H08680, H20556, H20650, H46154, H46155, R88298, R90733, R90759, R92224, R92332, R97325, H57663, H58503, H61709, H61913, H62747, H66685, H68924, H68954, H80053, H83342, H95786, H96135, N20464, N20472, N24026, N25491, N35235, N35419, N38769, N44900, N48399, N53146, N55089, N55095, N57767, N58580, N59732, N63942, N70290, N71759, N74938, N77300, N98411, W23555, W52690, W52160, W56557, W56635, W56598, W56594, W73408, W74230, W79843, W93916, AA031492, AA070868, AA071019, AA088788, AA100685, AA112926, AA176829, AA176851, AA193034, AA194065, AA194180, AA194579, AA194703, AA195416, AA195532, AA233792, AA233783, AA233900, AA233920, AA234128, AA234169, AA252704, AA252831, AA416743, AA418391, AA418440
772582	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 960 of SEQ ID NO:132, b is an integer of 15 to 974, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:132, and where b is greater than or equal to a + 14.	
773387	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 620 of SEQ ID NO:133, b is an integer of 15 to 634, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:133, and where b is greater than or equal to a + 14.	
773827	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1841 of SEQ ID NO:134.	

	b is an integer of 15 to 1855, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:134, and where b is greater than or equal to a + 14.	
774108	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 903 of SEQ ID NO:135, b is an integer of 15 to 917, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:135, and where b is greater than or equal to a + 14.	T96288, R31388, R32886, R63543, R63597, R75811, R75812, H20285, H20509, H20599, H21238, H24872, H29854, H29945, H41103, H41208, H44188, H44189, R85628, R91367, H83459, H83571, H97165, H97164, N25639, N29652, N29777, N32407, N32413, N32580, N32835, N41918, N42281, N56607, N57152, N57196, N69818, N70613, N93340, N93928, N94454, W24358, W25163, W30800, W37904, W37964, W40428, W68631, W68632, W70339, W80994, W81096, W81716, W81253, W81543, W81544, W94206, AA004372, AA011346, AA016002, AA028888, AA029626, AA029627, AA044028, AA044350, AA062804, AA081035, AA131270, AA131354, AA131371
774636	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1257 of SEQ ID NO:136, b is an integer of 15 to 1271, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:136, and where b is greater than or equal to a + 14.	T54747, T69827, R14146, R50592, R55502, R73615, R73937, H41540, R84981, R85103, R87495, R88553, R88554, R88556, R88818, R88839, R89675, R91235, H51003, H51004, H51581, H79057, N70799, W02680, AA232327, AA232417, AA464467
775339	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2003 of SEQ ID NO:137, b is an integer of 15 to 2017, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:137, and where b is greater than or equal to a + 14.	
775582	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 923 of SEQ ID NO:138, b is an integer of 15 to 937, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:138, and where b is greater than or	T62486, T62631, H14642, R85991, H73603, N54912, N68727, N80228, N91617, W38518, W67302, W67418, AA171395, AA214500, AA215291, AA464035



	equal to $a + 14$ .	
775779	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2745 of SEQ ID NO:139, b is an integer of 15 to 2759, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:139, and where b is greater than or equal to $a + 14$ .	
777809	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1227 of SEQ ID NO:140, b is an integer of 15 to 1241, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:140, and where b is greater than or equal to $a + 14$ .	
778927	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3391 of SEQ ID NO:141, b is an integer of 15 to 3405, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:141, and where b is greater than or equal to $a + 14$ .	T50777, T50939, R11800, R19713, R31403, R32898, R44269, R44269, R55431, R60041, R60103, R69554, R74340, R74434, H20427, H26615, H26660, H42495, H43482, R85644, H51488, H68618, N58157, N58231, N77611, W39692, W45048, W56828, W57633, AA052900, AA057808, AA074705, AA122120, AA121079, AA121231, AA259051, AA464470
779262	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2254 of SEQ ID NO:142, b is an integer of 15 to 2268, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:142, and where b is greater than or equal to $a + 14$ .	R11844, R71241, R71292, H00159, H88551, H90726, H98059, N28770, N58442, N78033, W32671, AA035075, AA112651, AA112652, AA130035, AA215309, AA251209
779392	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1743 of SEQ ID NO:143, b is an integer of 15 to 1757, where both	R25284, R36255, R36256, R42970, R46635, R42970, R46635, H28773, N52867, N70541, N77890, W05403, W05783, AA085067, AA085066, AA204650, AA210753, AA211713, AA251462, AA252456, AA460350, AA460780

	a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:143, and where b is greater than or equal to a + 14.	
780149	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1048 of SEQ ID NO:144, b is an integer of 15 to 1062, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:144, and where b is greater than or equal to a + 14.	
780583	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1016 of SEQ ID NO:145, b is an integer of 15 to 1030, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:145, and where b is greater than or equal to a + 14.	
780960	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 800 of SEQ ID NO:146, b is an integer of 15 to 814, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:146, and where b is greater than or equal to a + 14.	
781469	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2664 of SEQ ID NO:147, b is an integer of 15 to 2678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:147, and where b is greater than or equal to a + 14.	T95791, H18820, H19074, H22604, H40723, H45802, H46056, H47074, H47156, H86819, H86886, H88675, H88724, H88972, H89058, H88972, N28987, N36053, N39668, N47281, W19145, W68543, W68544, N91577, AA044679, AA044896, AA430011
781556	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	T94861, T94906, R21516, R26869, R27098, R36258, R37965, R37966, R78172, H03413, H04116, H14531, H45546, R96826, R98130, N51409, N52365, N64272, N74939, N75136,

	formula of a-b, where a is any integer between 1 to 1014 of SEQ ID NO:148, b is an integer of 15 to 1028, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:148, and where b is greater than or equal to a + 14.	W23556, W35208, AA187823, AA191525, AA429367
781771	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1411 of SEQ ID NO:149, b is an integer of 15 to 1425, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:149, and where b is greater than or equal to a + 14.	T95420, T99529, R50341, R52125, R72608, R72630, R72677, R72701, H26733, H26734, H30106, H59788, H82441, N75150, W42750, W42840
782033	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 766 of SEQ ID NO:150, b is an integer of 15 to 780, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:150, and where b is greater than or equal to a + 14.	H53100, H53207, H97410, H98035, N30753, N68541, W42491, W42641, W57808, AA046603, AA046753, AA136886, AA136997, AA143419, AA143420
782105	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1052 of SEQ ID NO:151, b is an integer of 15 to 1066, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:151, and where b is greater than or equal to a + 14.	R97486, H72940, W90139
782122	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1635 of SEQ ID NO:152, b is an integer of 15 to 1649, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:152, and where b is greater than or equal to a + 14.	T54379, T60348, T61029, T54271, T57801, R10793, T78907, T78959, R49078, R55635, R67844, R67845, R69587, R72600, R72666, H04742, H04830, H16978, H24654, H26129, H26308, H26395, H26467, H28100, H28205, H28252, H28895, H28896, H30485, H39554, H42595, H42603, H42662, H43740, H44345, H44346, H44546, H44547, H44960, H45012, H45860, R88120, R88214, H51204, H58080, H58081, H64553, H64654, H70033, H70034, H86451, H70034, H99833, N24525, N29867, N30752, N35500, N39259, N42463, N44804,

		N52550, N53985, N57289, N58726, N63349, N67624, N67663, N68157, N70299, N80615, N93230, N94595, N98489, W19633, W23803, W25087, W31034, W37981, W37982, W42579, W44389, W49677, W57614, W57871, W58142, W67781, W67840, W68147, W68474, W68699, W68791, W69717, W80749, W80837, N89879, AA025233, AA025568, AA025686, AA026020, AA033846, AA039625, AA039693, AA046842, AA047013, AA057608, AA057676, AA064637, AA064680, AA074448, AA083591, AA098837, AA102142, AA113374, AA113402, AA115525, AA114948, AA128972, AA128973, AA133142, AA146949, AA148086, AA149283, AA149377, AA160012, AA160688, AA172144, AA180932, AA182561
783135	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 646 of SEQ ID NO:153, b is an integer of 15 to 660, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:153, and where b is greater than or equal to a + 14.	
783245	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 591 of SEQ ID NO:154, b is an integer of 15 to 605, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:154, and where b is greater than or equal to a + 14.	
783247	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 681 of SEQ ID NO:155, b is an integer of 15 to 695, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:155, and where b is greater than or equal to a + 14.	AA155638
783413	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	H58751, H93683, H93684, N93167, W19186, W19958, W38771, N91367

	sequence described by the general formula of a-b, where a is any integer between 1 to 766 of SEQ ID NO:156, b is an integer of 15 to 780, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:156, and where b is greater than or equal to a + 14.	
784407	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1113 of SEQ ID NO:157, b is an integer of 15 to 1127, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:157, and where b is greater than or equal to a + 14.	
784548	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1268 of SEQ ID NO:158, b is an integer of 15 to 1282, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:158, and where b is greater than or equal to a + 14.	
785075	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1491 of SEQ ID NO:159, b is an integer of 15 to 1505, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:159, and where b is greater than or equal to a + 14.	
785677	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 722 of SEQ ID NO:160, b is an integer of 15 to 736, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:160, and where b is greater than or equal to a + 14.	

786238	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 981 of SEQ ID NO:161, b is an integer of 15 to 995, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:161, and where b is greater than or equal to a + 14.	
786389	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1111 of SEQ ID NO:162, b is an integer of 15 to 1125, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:162, and where b is greater than or equal to a + 14.	
786929	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 409 of SEQ ID NO:163, b is an integer of 15 to 423, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:163, and where b is greater than or equal to a + 14.	
786932	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1628 of SEQ ID NO:164, b is an integer of 15 to 1642, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:164, and where b is greater than or equal to a + 14.	
787078	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1101 of SEQ ID NO:165, b is an integer of 15 to 1115, where both a and b correspond to the positions of	

	nucleotide residues shown in SEQ ID NO:165, and where b is greater than or equal to a + 14.	
787139	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1052 of SEQ ID NO:166, b is an integer of 15 to 1066, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:166, and where b is greater than or equal to a + 14.	
787283	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 643 of SEQ ID NO:167, b is an integer of 15 to 657, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:167, and where b is greater than or equal to a + 14.	R22724
788761	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1012 of SEQ ID NO:168, b is an integer of 15 to 1026, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:168, and where b is greater than or equal to a + 14.	
788988	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 760 of SEQ ID NO:169, b is an integer of 15 to 774, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:169, and where b is greater than or equal to a + 14.	
789092	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	AA234588

	between 1 to 388 of SEQ ID NO:170, b is an integer of 15 to 402, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:170, and where b is greater than or equal to a + 14.	
789298	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 782 of SEQ ID NO:171, b is an integer of 15 to 796, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:171, and where b is greater than or equal to a + 14.	
789299	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 464 of SEQ ID NO:172, b is an integer of 15 to 478, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:172, and where b is greater than or equal to a + 14.	
789718	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 642 of SEQ ID NO:173, b is an integer of 15 to 656, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:173, and where b is greater than or equal to a + 14.	
789957	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1877 of SEQ ID NO:174, b is an integer of 15 to 1891, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:174, and where b is greater than or equal to a + 14.	T51260, T61941, T62167, T77034, T90753, R38108, N32708, N92379, W24621, W42543, W42478, AA128007, AA128031, AA134234, AA424998
789977	Preferably excluded from the present invention are one or more	T56442, T78292, R37940, R56008, R56009, R56573, R56574, H11080, N34431, N48665,



	polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2147 of SEQ ID NO:175, b is an integer of 15 to 2161, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:175, and where b is greater than or equal to a + 14.	AA010749, AA011177, AA070806, AA070882, AA146859, AA147636, AA147691, AA164223, AA164224, AA210729, AA210859, AA243063, AA243070, AA464493, AA464494
790285	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2397 of SEQ ID NO:176, b is an integer of 15 to 2411, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:176, and where b is greater than or equal to a + 14.	T66279, T66328, T84164, T85098, R24232, R24233, H03657, H03658, H98526, H98556, H99618, N22728, N29400, N32172, N33953, N41460, N69471, N70552, N73722, W03893, W44579, W72407, W76486, W78102, W79410, N90963, AA044816, AA044841, AA086039, AA086121, AA088877, AA102298, AA130887, AA131529, AA131603, AA181784, AA182515, AA190450, AA191392, AA223757
790509	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1324 of SEQ ID NO:177, b is an integer of 15 to 1338, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:177, and where b is greater than or equal to a + 14.	T68040, H17760, AA101036, AA129837
790775	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1600 of SEQ ID NO:178, b is an integer of 15 to 1614, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:178, and where b is greater than or equal to a + 14.	N25320, N31432, W81044, W81097
790888	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4278 of SEQ ID NO:179, b is an integer of 15 to 4292, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:179, and where b is greater than or	R14550, R15204, T26493, R21597, R22908, R23010, R41211, R41649, R43371, R41211, R41649, R43371, R58989, R59048, H05739, H05845, H17266, H17265, H23579, H44104, H46505, H47043, H58955, H59002, H73676, H73730, H80078, H82275, H82289, H82399, H82381, H97810, H98133, H98737, N23117, N24310, N25196, N25265, N27792, N28735, N29893, N33395, N33904, N36066, N36839, N42542, N46060, N51230, N59535, N67737,

	equal to $a + 14$ .	N73641, N78481, N78694, W03555, W15202, W52445, W52723, W95124, AA047257, AA057142, AA204699, AA251464, AA430598
791506	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 229 of SEQ ID NO:180, b is an integer of 15 to 243, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:180, and where b is greater than or equal to $a + 14$ .	
791649	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 799 of SEQ ID NO:181, b is an integer of 15 to 813, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:181, and where b is greater than or equal to $a + 14$ .	
791802	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 808 of SEQ ID NO:182, b is an integer of 15 to 822, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:182, and where b is greater than or equal to $a + 14$ .	
792002	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1081 of SEQ ID NO:183, b is an integer of 15 to 1095, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:183, and where b is greater than or equal to $a + 14$ .	T49735, T49736, T95310, T95391, T99384, T99612, R63493, R63494, H27739, R91698, R92136, H52608, H57619, H58464, H61415, H62139, H69019, H87167, H87669, N21358, N70307, N79596, W19063, W58498, W58651, W79687, W81289, AA099849, AA099972, AA232767
792291	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	T55436, R21797, R22403, R22452, R22916, R23020, R76901, R77068, H22573, H25752, H25866, R83900, H50717, H50821, H64026, H64791, H95702, N64545, N69769, N74704, N80341, W05092, W79489, W79634,

	between 1 to 3661 of SEQ ID NO:184, b is an integer of 15 to 3675, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:184, and where b is greater than or equal to a + 14.	AA005055, AA005007, AA025043, AA036711, AA037127, AA043916, AA055100, AA063627, AA069142, AA069230, AA069323, AA069376, AA112277, AA112531, AA115279, AA151238, AA151239, AA151582, AA149398, AA149961, AA150069, AA158029, AA158321, AA158692, AA158693, AA161232, AA236787, AA236834, AA256776, AA261961
792371	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1026 of SEQ ID NO:185, b is an integer of 15 to 1040, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:185, and where b is greater than or equal to a + 14.	
792660	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 803 of SEQ ID NO:186, b is an integer of 15 to 817, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:186, and where b is greater than or equal to a + 14.	T59054, T86590, T83271, R48677, R53483, R53482, R62329, R62330, R66651, R67372, R69095, R69210, R71144, R82632, R82676, H15764, H15765, H19518, H19605, H27898, H42872, H42936, H49329, H49330, H50062, H50061, H87268, H87324, H96667, N22675, N92574, W37223, W37563, W38866, W61119, W65380, AA035095, AA035635, AA037254, AA054951, AA062973, AA082301, AA132472
792782	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1066 of SEQ ID NO:187, b is an integer of 15 to 1080, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:187, and where b is greater than or equal to a + 14.	
792890	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1272 of SEQ ID NO:188, b is an integer of 15 to 1286, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:188, and where b is greater than or equal to a + 14.	AA251351

792931	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1724 of SEQ ID NO:189, b is an integer of 15 to 1738, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:189, and where b is greater than or equal to a + 14.	
792943	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1909 of SEQ ID NO:190, b is an integer of 15 to 1923, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:190, and where b is greater than or equal to a + 14.	
793104	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 236 of SEQ ID NO:191, b is an integer of 15 to 250, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:191, and where b is greater than or equal to a + 14.	
793445	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1888 of SEQ ID NO:192, b is an integer of 15 to 1902, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:192, and where b is greater than or equal to a + 14.	AA034998, AA044249, AA088830, AA429418
793446	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 546 of SEQ ID NO:193, b is an integer of 15 to 560, where both a and b correspond to the positions of	T57765, T60664, H01264, H45774, H54790, H54842, H64484, H64485, N98810, W58332, W58653, W74582, W79320, W79420, W79565, W92452, AA027210, AA027209, AA029725, AA029663, AA088693, AA121506, AA127731, AA428362

	nucleotide residues shown in SEQ ID NO:193, and where b is greater than or equal to a + 14.	
793639	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 576 of SEQ ID NO:194, b is an integer of 15 to 590, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:194, and where b is greater than or equal to a + 14.	N69881, N93023, N98853, W21375, W73944, W77988, AA169530, AA169837, AA176453, AA176931
794213	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 677 of SEQ ID NO:195, b is an integer of 15 to 691, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:195, and where b is greater than or equal to a + 14.	N53897, N55318
795858	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1758 of SEQ ID NO:196, b is an integer of 15 to 1772, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:196, and where b is greater than or equal to a + 14.	
795955	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 661 of SEQ ID NO:197, b is an integer of 15 to 675, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:197, and where b is greater than or equal to a + 14.	
796359	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	

	between 1 to 543 of SEQ ID NO:198, b is an integer of 15 to 557, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:198, and where b is greater than or equal to $a + 14$ .	
796555	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2597 of SEQ ID NO:199, b is an integer of 15 to 2611, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:199, and where b is greater than or equal to $a + 14$ .	T69136, T69194, T95612, T95713, R53091, R73126, N41876, N49174, W05348, W04725, W31397, W31827, W92674, AA039513
796675	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2302 of SEQ ID NO:200, b is an integer of 15 to 2316, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:200, and where b is greater than or equal to $a + 14$ .	
796743	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1133 of SEQ ID NO:201, b is an integer of 15 to 1147, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:201, and where b is greater than or equal to $a + 14$ .	
796792	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 674 of SEQ ID NO:202, b is an integer of 15 to 688, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:202, and where b is greater than or equal to $a + 14$ .	
799668	Preferably excluded from the present invention are one or more	

	<p>polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 290 of SEQ ID NO:203, b is an integer of 15 to 304, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:203, and where b is greater than or equal to a + 14.</p>	
799669	<p>Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 403 of SEQ ID NO:204, b is an integer of 15 to 417, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:204, and where b is greater than or equal to a + 14.</p>	
799673	<p>Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 537 of SEQ ID NO:205, b is an integer of 15 to 551, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:205, and where b is greater than or equal to a + 14.</p>	
799674	<p>Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1087 of SEQ ID NO:206, b is an integer of 15 to 1101, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:206, and where b is greater than or equal to a + 14.</p>	
799678	<p>Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 501 of SEQ ID NO:207, b is an integer of 15 to 515, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:207, and where b is greater than or</p>	

	equal to $a + 14$ .	
799728	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 255 of SEQ ID NO:208, b is an integer of 15 to 269, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:208, and where b is greater than or equal to $a + 14$ .	
799748	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 720 of SEQ ID NO:209, b is an integer of 15 to 734, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:209, and where b is greater than or equal to $a + 14$ .	H19497, H19579, H50117, H50164, H52826, H52827, H61184, H62087, H96290, H96291, N20586, N21261, N28978, N30137, N30490, N35750, W31933, W37535, N90542, AA418545, AA418511
799760	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 644 of SEQ ID NO:210, b is an integer of 15 to 658, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:210, and where b is greater than or equal to $a + 14$ .	
799805	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 190 of SEQ ID NO:211, b is an integer of 15 to 204, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:211, and where b is greater than or equal to $a + 14$ .	
800296	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1257 of SEQ ID NO:212, b is an integer of 15 to 1271, where both	



	a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:212, and where b is greater than or equal to a + 14.	
800327	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1011 of SEQ ID NO:213, b is an integer of 15 to 1025, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:213, and where b is greater than or equal to a + 14.	
800816	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 337 of SEQ ID NO:214, b is an integer of 15 to 351, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:214, and where b is greater than or equal to a + 14.	
800835	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1073 of SEQ ID NO:215, b is an integer of 15 to 1087, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:215, and where b is greater than or equal to a + 14.	
805429	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1963 of SEQ ID NO:216, b is an integer of 15 to 1977, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:216, and where b is greater than or equal to a + 14.	
805458	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	T82438, T82439, R19121, R20391, R28602, R36743, R43508, R46035, R43508, R46035, R79588, H24625, N28372, N28785, N29421, N35476, N57353, N72836, N79096, W03034.

	formula of a-b, where a is any integer between 1 to 2801 of SEQ ID NO:217, b is an integer of 15 to 2815, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:217, and where b is greater than or equal to a + 14.	AA016073, AA019733, AA021030, AA062895, AA081968, AA115692, AA133511, AA151852, AA149707, AA194903, AA194902
805478	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1631 of SEQ ID NO:218, b is an integer of 15 to 1645, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:218, and where b is greater than or equal to a + 14.	
805805	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 464 of SEQ ID NO:219, b is an integer of 15 to 478, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:219, and where b is greater than or equal to a + 14.	
806486	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 818 of SEQ ID NO:220, b is an integer of 15 to 832, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:220, and where b is greater than or equal to a + 14.	
806498	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1878 of SEQ ID NO:221, b is an integer of 15 to 1892, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:221, and where b is greater than or equal to a + 14.	
806819	Preferably excluded from the present	

	invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 854 of SEQ ID NO:222, b is an integer of 15 to 868, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:222, and where b is greater than or equal to a + 14.	
810870	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1502 of SEQ ID NO:223, b is an integer of 15 to 1516, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:223, and where b is greater than or equal to a + 14.	R50267, R50730, H27672, H27673, H30138, H99256, N74342, N80868, W05054, W07601
811730	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1292 of SEQ ID NO:224, b is an integer of 15 to 1306, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:224, and where b is greater than or equal to a + 14.	
813025	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 570 of SEQ ID NO:225, b is an integer of 15 to 584, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:225, and where b is greater than or equal to a + 14.	
813233	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 509 of SEQ ID NO:226, b is an integer of 15 to 523, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	

	NO:226, and where b is greater than or equal to a + 14.	
813262	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2363 of SEQ ID NO:227, b is an integer of 15 to 2377, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:227, and where b is greater than or equal to a + 14.	
815637	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 449 of SEQ ID NO:228, b is an integer of 15 to 463, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:228, and where b is greater than or equal to a + 14.	
815853	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1218 of SEQ ID NO:229, b is an integer of 15 to 1232, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:229, and where b is greater than or equal to a + 14.	R53293, R59708, R59818, R88929, R89609, H78819, N52182, AA125808, AA128281
815999	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1049 of SEQ ID NO:230, b is an integer of 15 to 1063, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:230, and where b is greater than or equal to a + 14.	
823427	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1049 of SEQ ID NO:231.	T53986, T60846, T72425, R18752, H22479, H50211, N40817, N93431, W21474, W21308, W32281, W44860, W95821, N90881, AA132037, AA131965, AA151157, AA155868, AA156600, AA156837, AA157061, AA157045, AA160623, AA169460, AA176447, AA178894,

	b is an integer of 15 to 1063, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:231, and where b is greater than or equal to a + 14.	AA179764, AA180438, AA181145, AA181144, AA196382, AA196478
823704	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1460 of SEQ ID NO:232, b is an integer of 15 to 1474, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:232, and where b is greater than or equal to a + 14.	
824798	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1768 of SEQ ID NO:233, b is an integer of 15 to 1782, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:233, and where b is greater than or equal to a + 14.	
825018	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2194 of SEQ ID NO:234, b is an integer of 15 to 2208, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:234, and where b is greater than or equal to a + 14.	
825076	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2566 of SEQ ID NO:235, b is an integer of 15 to 2580, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:235, and where b is greater than or equal to a + 14.	
825787	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	

	sequence described by the general formula of a-b, where a is any integer between 1 to 2994 of SEQ ID NO:236, b is an integer of 15 to 3008, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:236, and where b is greater than or equal to a + 14.	
826116	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 863 of SEQ ID NO:237, b is an integer of 15 to 877, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:237, and where b is greater than or equal to a + 14.	
826147	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3025 of SEQ ID NO:238, b is an integer of 15 to 3039, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:238, and where b is greater than or equal to a + 14.	
827020	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1978 of SEQ ID NO:239, b is an integer of 15 to 1992, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:239, and where b is greater than or equal to a + 14.	
827586	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 483 of SEQ ID NO:240, b is an integer of 15 to 497, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:240, and where b is greater than or equal to a + 14.	

827732	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 302 of SEQ ID NO:241, b is an integer of 15 to 316, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:241, and where b is greater than or equal to a + 14.	
827735	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 815 of SEQ ID NO:242, b is an integer of 15 to 829, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:242, and where b is greater than or equal to a + 14.	
827740	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 824 of SEQ ID NO:243, b is an integer of 15 to 838, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:243, and where b is greater than or equal to a + 14.	R21513, R22316, R42033, R43706, R42033, R43706, R63113, R70954, R71006, N48618, N53377, AA912400
827808	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2839 of SEQ ID NO:244, b is an integer of 15 to 2853, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:244, and where b is greater than or equal to a + 14.	
828251	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1183 of SEQ ID NO:245, b is an integer of 15 to 1197, where both a and b correspond to the positions of	

	nucleotide residues shown in SEQ ID NO:245, and where b is greater than or equal to a + 14.	
828357	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 834 of SEQ ID NO:246, b is an integer of 15 to 848, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:246, and where b is greater than or equal to a + 14.	
828449	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1322 of SEQ ID NO:247, b is an integer of 15 to 1336, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:247, and where b is greater than or equal to a + 14.	
828612	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1062 of SEQ ID NO:248, b is an integer of 15 to 1076, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:248, and where b is greater than or equal to a + 14.	R28513, R28661, R31336, R41867, R41867, R60004, H19945, H19946, H22061, H46271, H46342, H82619, H82618, N20678, W96169, AA010842, AA278855, AA582295, AA583721, AA639735, AA579409, AA568321, AA833752, AA907437, AI054389, W22584
828647	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2411 of SEQ ID NO:249, b is an integer of 15 to 2425, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:249, and where b is greater than or equal to a + 14.	
828698	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	



	between 1 to 1394 of SEQ ID NO:250, b is an integer of 15 to 1408, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:250, and where b is greater than or equal to a + 14.	
828962	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 480 of SEQ ID NO:251, b is an integer of 15 to 494, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:251, and where b is greater than or equal to a + 14.	
828982	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2477 of SEQ ID NO:252, b is an integer of 15 to 2491, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:252, and where b is greater than or equal to a + 14.	T64550, T65973, T94849, T94894, R07359, R07409, R34782, R35670, R35781, R56137, R56532, R64039, R66397, R67131, H01215, H02256, H02354, H03227, H04019, R94572, R94573, H51242, H60286, H65939, H72416, H72857, N22537, N24628, N24936, N33813, N35712, N35830, N35916, N43982, N51363, N64462, N70838, N75470, N75760, W01444, W05279, W57605, W58752, W72612, W72970, W73260, W73535, W76678, W76207, W94918, W91971, W92319, W92355, AA024690, AA024643, AA028083, AA028084, AA028169, AA035743, AA045830, AA045917, AA081723, AA086310, AA085740, AA102651, AA101305, AA126788, AA126837, AA126865, AA127295, AA129688, AA129664, AA133503, AA133504, AA132801, AA134537, AA134547, AA186712, AA188264, AA215597, AA463977, AA464112, AA417286, AA417312, AA259228, AA279952, AA287814, AA468227, AA468302, AA526480, AA553703, AA587072, AA635683, AA639361, AA573471, AA579754, AA579812, AA580600, AA730425, AA741436, AA804629, AA829189, AA830255, AA865594, AA885821, AA918979, AA962033, AA985542, AA985571, AA987607, AA995783, A1075334, D79160, N84712, N88655, C03235, AA094028
829282	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1111 of SEQ ID NO:253, b is an integer of 15 to 1125, where both a and b correspond to the positions of	

	nucleotide residues shown in SEQ ID NO:253, and where b is greater than or equal to a + 14.	
829368	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1395 of SEQ ID NO:254, b is an integer of 15 to 1409, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:254, and where b is greater than or equal to a + 14.	R61547, R76124, H01565, H02950, H04248, H29996, H99672, W19970
829751	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 476 of SEQ ID NO:255, b is an integer of 15 to 490, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:255, and where b is greater than or equal to a + 14.	
829773	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1219 of SEQ ID NO:256, b is an integer of 15 to 1233, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:256, and where b is greater than or equal to a + 14.	T96982, T97094, H53488, H53861, H64894, H65486, N62304, N67480, N78709, W03409, W07598, W73770, AA025496, AA025812, AA133948
829934	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2390 of SEQ ID NO:257, b is an integer of 15 to 2404, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:257, and where b is greater than or equal to a + 14.	
829942	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	T64541, T65964, R01423, R01424, R05277, R19450, R44699, R51779, R51780, R44699, H11322, H11349, H13859, H113911, H21393, H21437, H21890, H22117, H45982, H46047, H47137, R98886, H54491, H54854, H98744,

	between 1 to 2078 of SEQ ID NO:258, b is an integer of 15 to 2092, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:258, and where b is greater than or equal to a + 14.	N23465, N37080, N46155, N46396, N58995, N62715, N93640, W60228, W60227, W74349, W76544, W87768, W87883, W90517, W90518, AA010775, AA011055, AA029083, AA029084, AA036822, AA057660, AA075916, AA082814, AA101057, AA130702, AA132788, AA133063, AA147813, AA148063, AA151487, AA151511, AA173298, AA173348, AA181036, AA187993, AA187994, AA192370, AA192357, AA243010, AA243264, AA250948
829951	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 373 of SEQ ID NO:259, b is an integer of 15 to 387, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:259, and where b is greater than or equal to a + 14.	
830173	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3698 of SEQ ID NO:260, b is an integer of 15 to 3712, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:260, and where b is greater than or equal to a + 14.	T52493, T52572, T56913, T61268, T61320, T70063, T70130, T72005, T87844, T94182, T70248, R24534, R24639, R31200, R64161, R64274, R70751, R70750, H16189, H89274, H99749, N25430, N25537, N32578, N32816, N34120, N34134, N34491, N35081, N42260, N43821, N62152, N62798, N64065, N64169, N67362, N69808, N74678, N93912, N49165, W04704, W05040, W16565, W19920, W31806, W31907, W37354, W37355, W40493, W45266, W45455, W52925, W58628, W92222, W92345, N91265, AA027083, AA027124, AA028969, AA029137, AA029257, AA083657, AA084297, AA121151, AA121131, AA126957, AA127166, AA128353, AA128495, AA128834, AA132690, AA132783, AA136553, AA152414, AA150706, AA150808, AA156272, AA164766, AA164767, AA171427, AA171794, AA173592, AA173949, AA190421, AA190580, AA191383, AA224415, AA232135
830200	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 883 of SEQ ID NO:261, b is an integer of 15 to 897, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:261, and where b is greater than or equal to a + 14.	AA524284, AA662477, AA887924

830365	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1891 of SEQ ID NO:262, b is an integer of 15 to 1905, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:262, and where b is greater than or equal to a + 14.	R42905, R59718, R62419, R72182, R72228, H22520, H22519, H25889, H45643, H46451, H46992, H84483, N50834, N92573, AA022699, AA022791, AA037734, AA037735, AA040585, AA040557, AA047816, AA159187, AA159282, AA223337, AA505391, AA515591, AA524466, AA613383, AA627298, AA578816, AA769153, AA826456, AA830896, AA831083, AA837917, AA977053, A1083822, A1090301, A1084104
830456	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1410 of SEQ ID NO:263, b is an integer of 15 to 1424, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:263, and where b is greater than or equal to a + 14.	T39800, T39875, T40331, T80148, R01135, R05754, R12866, R15287, R21703, R39361, H00652, H00741, H05366, H17706, H23423, R97800, R97849, N25478, N41797, N48511, N98906, W19893, W23945, W35174, W60540, W78229, W79282, W84685, AA022952, AA026821, AA026953, AA074956, AA075111, AA114974, AA114988, AA192860, AA193064
830549	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1273 of SEQ ID NO:264, b is an integer of 15 to 1287, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:264, and where b is greater than or equal to a + 14.	R60171, H26796, H96303, N91699, W25137, AA069218, AA088565, AA161178
830602	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 977 of SEQ ID NO:265, b is an integer of 15 to 991, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:265, and where b is greater than or equal to a + 14.	
830610	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2306 of SEQ ID NO:266, b is an integer of 15 to 2320, where both a and b correspond to the positions of	

	nucleotide residues shown in SEQ ID NO:266, and where b is greater than or equal to a + 14.	
830644	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 409 of SEQ ID NO:267, b is an integer of 15 to 423, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:267, and where b is greater than or equal to a + 14.	
830707	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1832 of SEQ ID NO:268, b is an integer of 15 to 1846, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:268, and where b is greater than or equal to a + 14.	
830709	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 587 of SEQ ID NO:269, b is an integer of 15 to 601, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:269, and where b is greater than or equal to a + 14.	
830733	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 866 of SEQ ID NO:270, b is an integer of 15 to 880, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:270, and where b is greater than or equal to a + 14.	T26638, R49962, H96664, N71762, N90691, AA040156, AA128271, AA418045, AA418216, AA535799, AA583405, AA768811
830768	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	

	between 1 to 2470 of SEQ ID NO:271, b is an integer of 15 to 2484, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:271, and where b is greater than or equal to a + 14.	
830855	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 737 of SEQ ID NO:272, b is an integer of 15 to 751, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:272, and where b is greater than or equal to a + 14.	H17127, AA100311, AA112910, AA282249, AA578649, AA748590
830949	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3295 of SEQ ID NO:273, b is an integer of 15 to 3309, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:273, and where b is greater than or equal to a + 14.	
830965	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 829 of SEQ ID NO:274, b is an integer of 15 to 843, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:274, and where b is greater than or equal to a + 14.	
830973	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2014 of SEQ ID NO:275, b is an integer of 15 to 2028, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:275, and where b is greater than or equal to a + 14.	
830979	Preferably excluded from the present invention are one or more	

	polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1441 of SEQ ID NO:276, b is an integer of 15 to 1455, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:276, and where b is greater than or equal to a + 14.	
830989	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1909 of SEQ ID NO:277, b is an integer of 15 to 1923, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:277, and where b is greater than or equal to a + 14.	
831134	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1366 of SEQ ID NO:278, b is an integer of 15 to 1380, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:278, and where b is greater than or equal to a + 14.	
831200	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1004 of SEQ ID NO:279, b is an integer of 15 to 1018, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:279, and where b is greater than or equal to a + 14.	
831260	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1178 of SEQ ID NO:280, b is an integer of 15 to 1192, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:280, and where b is greater than or	R15008, R28066, R68324, H20638, N25438, N67982, N67983, N67999, N68004, N68005, N80403, N80423, N80429, N80430, AA024581, AA024582, AA024637, AA862760, AA091142

	equal to $a + 14$ .	
831531	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1741 of SEQ ID NO:281, b is an integer of 15 to 1755, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:281, and where b is greater than or equal to $a + 14$ .	T66624, R16038, R26139, R26353, H15795, H16285, H21749, H21945, H22698, H23978, H52286, H52523, H60184, H60227, H68044, H81748, H81749, N46859, N47179, N51722, N51808, AA031701, AA031866, AA043760, AA043761, AA081005, AA081148, AA195519, AA470636, AA534463, AA555198, AA631348, AA721036, AA737025, AA761301, AA764993, AA765314, AA765749, AA878422, U47720, C21223
831665	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1079 of SEQ ID NO:282, b is an integer of 15 to 1093, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:282, and where b is greater than or equal to $a + 14$ .	
831724	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1542 of SEQ ID NO:283, b is an integer of 15 to 1556, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:283, and where b is greater than or equal to $a + 14$ .	R52161, N45179, N68350, N94021, W02782, W24840, W61323, AA907441
831884	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1015 of SEQ ID NO:284, b is an integer of 15 to 1029, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:284, and where b is greater than or equal to $a + 14$ .	
831897	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1569 of SEQ ID NO:285, b is an integer of 15 to 1583, where both	AA056348, AA127534



	a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:285, and where b is greater than or equal to $a + 14$ .	
831922	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1163 of SEQ ID NO:286, b is an integer of 15 to 1177, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:286, and where b is greater than or equal to $a + 14$ .	
831963	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 492 of SEQ ID NO:287, b is an integer of 15 to 506, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:287, and where b is greater than or equal to $a + 14$ .	
832074	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 934 of SEQ ID NO:288, b is an integer of 15 to 948, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:288, and where b is greater than or equal to $a + 14$ .	
832266	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1020 of SEQ ID NO:289, b is an integer of 15 to 1034, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:289, and where b is greater than or equal to $a + 14$ .	T70612, T70879, H13555, H23264, R97792, R97842, N75850, W07434, W19866, N90056, AA043395, AA463232, AA463231
832309	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	

	formula of a-b, where a is any integer between 1 to 3077 of SEQ ID NO:290, b is an integer of 15 to 3091, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:290, and where b is greater than or equal to a + 14.	
832342	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 504 of SEQ ID NO:291, b is an integer of 15 to 518, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:291, and where b is greater than or equal to a + 14.	
832351	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 484 of SEQ ID NO:292, b is an integer of 15 to 498, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:292, and where b is greater than or equal to a + 14.	
832352	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 455 of SEQ ID NO:293, b is an integer of 15 to 469, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:293, and where b is greater than or equal to a + 14.	
832434	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 654 of SEQ ID NO:294, b is an integer of 15 to 668, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:294, and where b is greater than or equal to a + 14.	
832490	Preferably excluded from the present	T86496, H24346, R84505, N26874, N98621,

	invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1386 of SEQ ID NO:295, b is an integer of 15 to 1400, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:295, and where b is greater than or equal to a + 14.	W04678, W04692, W24267, W93387, W94971, AA036953, AA136869, AA136799, AA147214, AA160413, AA535592, AA931261, AA931403, AA962726, AA992456
832573	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 946 of SEQ ID NO:296, b is an integer of 15 to 960, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:296, and where b is greater than or equal to a + 14.	
832580	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 643 of SEQ ID NO:297, b is an integer of 15 to 657, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:297, and where b is greater than or equal to a + 14.	
833394	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 878 of SEQ ID NO:298, b is an integer of 15 to 892, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:298, and where b is greater than or equal to a + 14.	
835355	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1610 of SEQ ID NO:299, b is an integer of 15 to 1624, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	AA076638, AA916592, A1088936, A1089690

	NO:299, and where b is greater than or equal to a + 14.	
835497	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1955 of SEQ ID NO:300, b is an integer of 15 to 1969, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:300, and where b is greater than or equal to a + 14.	
835728	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1868 of SEQ ID NO:301, b is an integer of 15 to 1882, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:301, and where b is greater than or equal to a + 14.	
835978	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2790 of SEQ ID NO:302, b is an integer of 15 to 2804, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:302, and where b is greater than or equal to a + 14.	
836091	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3845 of SEQ ID NO:303, b is an integer of 15 to 3859, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:303, and where b is greater than or equal to a + 14.	R02093, R02205, R02336, R02439, R19436, R44685, R44685, R72354, H10160, H49884, H49885, N23208, N28789, N29901, N42953, N55093, N77305, N99373, W46396, W46504, AA082311, AA176281, AA176282, AA227971, AA228079, AA234964, AA234145, AA281787, AA281656, AA524468, AA551888, AA631173, AA639499, AA811344, AA830439, AA831974, AA923665, C03439, AA641655, AA091346, AA400968, AA400884
836274	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3364 of SEQ ID NO:304,	T75442, R20393, R43511, R43511, R73650, R73731, R80152, R80886, H97932, H98616, N33018, N71679, N99650, AA001053, AA001089, AA044947, AA044943, AA149057, AA464856, AA427892, AA228265, AA230021, AA482694, AA483691, AA484850, AA513037,

	b is an integer of 15 to 3378, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:304, and where b is greater than or equal to a + 14.	AA516076, AA532381, AA583355, AA618566, AA577028, AA730651, AA730790, AA745667, AA829807, AA923038, AA931937, AA932867, AA934400, AA934413, AA971551, AA971743, AA972772, AA977253, AA992454, AA994794, AI089906, AI094921, D79281, C06099, D44840, C20741, AA283186, AA292346, AA394164
836731	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1000 of SEQ ID NO:305, b is an integer of 15 to 1014, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:305, and where b is greater than or equal to a + 14.	
838014	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2113 of SEQ ID NO:306, b is an integer of 15 to 2127, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:306, and where b is greater than or equal to a + 14.	
838874	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 652 of SEQ ID NO:307, b is an integer of 15 to 666, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:307, and where b is greater than or equal to a + 14.	R61165, N44200
839120	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2157 of SEQ ID NO:308, b is an integer of 15 to 2171, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:308, and where b is greater than or equal to a + 14.	T74462, R18264, H23432, AA279685, AA847441, AA904076, AA393782

839611	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 6149 of SEQ ID NO:309, b is an integer of 15 to 6163, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:309, and where b is greater than or equal to a + 14.	T93695, T93696, T96161, R32227, R32254, R32304, R33503, R34044, R71178, H93366, N50709, N55039, AA165143, AA199856, AA199927, AA234331, AA262892, AA423987, AA423986, AA525886, AA661602, AA731504, AA741228, AA814795, AA828858, AA829196, AA831198, AA834822, AA865590, AA886436, AA903649, D82270, D82453, D82464, AA642466, AA219620, AA219628, AA400707, AA400674, AA421941, AA633988, AA663219, AA663250, AA665538, AA724260, A1074714, T26891, T26926
840138	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2072 of SEQ ID NO:310, b is an integer of 15 to 2086, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:310, and where b is greater than or equal to a + 14.	
840616	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2149 of SEQ ID NO:311, b is an integer of 15 to 2163, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:311, and where b is greater than or equal to a + 14.	
840780	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1383 of SEQ ID NO:312, b is an integer of 15 to 1397, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:312, and where b is greater than or equal to a + 14.	
840857	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4092 of SEQ ID NO:313, b is an integer of 15 to 4106, where both	T50389, T50520, T55419, T55495, T55974, T57220, R34591, R34592, R69726, H21148, R85777, R99233, H61311, H62351, H85185, H88299, N23288, N32662, N58504, N78093, N92665, N99611, AA005068, AA007333, AA007334, AA036884, AA044715, AA045458, AA046500, AA045654, AA115936, AA121004,

	a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:313, and where b is greater than or equal to a + 14.	AA126775, AA133605, AA133606, AA133980, AA181633, AA182611, AA232979, AA233365, AA459953, AA460042, AA282826, AA285050, AA506082, AA558006, AA601060, AA767799, AA804323, AA807029, AA807087, AA825536, AA833810, AA922732, AA928638, AA960990, N56482, N62047, W27456, W26569, AA092778, AA652535, AA065256, AA065257, AA450197, AA452846, AA452986, AA705224, Z19460, AA884767, AA969488, AA977494, AI002996, AI032008, Z28526, D20112, T19336
840862	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 518 of SEQ ID NO:314, b is an integer of 15 to 532, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:314, and where b is greater than or equal to a + 14.	T94528, N40545, N46592, N92934, AA570273, AA873604, AA910827, AA932397, AA971868, AI095210, N56229, AA648290, F20835, AA629912
840864	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1924 of SEQ ID NO:315, b is an integer of 15 to 1938, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:315, and where b is greater than or equal to a + 14.	R40870, R44820, H26640, W78814, W80713, AA195492, AA937549, AI085492, AI094865, AA449317, AA884600, AA909529, AA923452, AA971781, AI084795, AI089007, AA702758, AA702769
840936	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 804 of SEQ ID NO:316, b is an integer of 15 to 818, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:316, and where b is greater than or equal to a + 14.	
840938	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 823 of SEQ ID NO:317, b is an integer of 15 to 837, where both a and b correspond to the positions of	

	nucleotide residues shown in SEQ ID NO:317, and where b is greater than or equal to a + 14.	
841884	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1434 of SEQ ID NO:318, b is an integer of 15 to 1448, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:318, and where b is greater than or equal to a + 14.	
842241	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1479 of SEQ ID NO:319, b is an integer of 15 to 1493, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:319, and where b is greater than or equal to a + 14.	
843712	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 595 of SEQ ID NO:320, b is an integer of 15 to 609, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:320, and where b is greater than or equal to a + 14.	R02291, N94598, W85882, AA255975
844040	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 488 of SEQ ID NO:321, b is an integer of 15 to 502, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:321, and where b is greater than or equal to a + 14.	W24428, AA143434, AA459809
844336	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer	



	between 1 to 2616 of SEQ ID NO:322, b is an integer of 15 to 2630, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:322, and where b is greater than or equal to a + 14.	
844612	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1860 of SEQ ID NO:323, b is an integer of 15 to 1874, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:323, and where b is greater than or equal to a + 14.	
844617	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2311 of SEQ ID NO:324, b is an integer of 15 to 2325, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:324, and where b is greater than or equal to a + 14.	
845251	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 771 of SEQ ID NO:325, b is an integer of 15 to 785, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:325, and where b is greater than or equal to a + 14.	T68474, AA159183, AA464447, AA424290, AA424487, AA631793, AA928390, AA946921, AA975194, AA977141, AA430527, AA430612, AA477798
845764	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 230 of SEQ ID NO:326, b is an integer of 15 to 244, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:326, and where b is greater than or equal to a + 14.	
846187	Preferably excluded from the present invention are one or more	

	<p>polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2440 of SEQ ID NO:327, b is an integer of 15 to 2454, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:327, and where b is greater than or equal to a + 14.</p>	
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### ***Polynucleotide and Polypeptide Variants***

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, and/or the cDNA sequence contained in a cDNA clone contained in the deposit.

5       The present invention also encompasses variants of the breast, ovarian, breast cancer and/or ovarian cancer polypeptide sequence disclosed in SEQ ID NO:Y, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X, and/or a polypeptide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

10       "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

15       The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the related cDNA contained in a deposited library or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise or alternatively consist of, a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, 25       under low stringency conditions, to the nucleotide coding sequence in SEQ ID NO:X, the

	residues: Glu-8 to Ser-15, Gly-42 to Leu-49, Pro-73 to Gly-79, Tyr-82 to Arg-87, Ser-109 to Gly-118, Glu-122 to Ile-128, Asp-132 to Gly-137, Asp-146 to Arg-151, Pro-153 to Lys-158, Gly-191 to His-197, Tyr-210 to Ser-218, Lys-234 to Gly-239, Ala-246 to Ala-252, His-257 to Pro-268, Ser-274 to Gly-280, Pro-316 to Tyr-323, Ile-358 to Leu-363, Gln-375 to Tyr-381, Gln-390 to Tyr-397, Gln-418 to Cys-430.
835497	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 718 as residues: Glu-141 to Pro-151, Asp-179 to Glu-184, Gly-214 to Ser-219, Thr-226 to Tyr-231, Thr-239 to Gly-248, Pro-281 to Gly-297, Pro-326 to Arg-336, Gln-408 to Asp-416.
835978	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 720 as residues: Trp-25 to Val-31.
836274	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 722 as residues: Ser-1 to Glu-9.
836731	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 723 as residues: Lys-15 to Glu-22, Gly-25 to Ala-34, Glu-75 to Gly-81, Gln-91 to Val-100, Pro-146 to Glu-155, Gln-161 to Phe-167, Asn-170 to Gly-178.
838014	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 724 as residues: Arg-1 to Pro-10, Asp-170 to Pro-176, Arg-203 to Tyr-212, Gly-228 to Lys-235.
838874	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 725 as residues: Gln-30 to Gln-45.
839120	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 726 as residues: Thr-22 to Arg-27, Arg-69 to Gly-75, Leu-77 to Pro-85.
839611	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 727 as residues: Asp-12 to Thr-17.
840138	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 728 as residues: Ser-1 to Thr-10.
840616	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 729 as residues: Lys-93 to Gly-99, Glu-144 to Leu-160, Ser-265 to Asp-270, Thr-382 to Gln-396, Val-512 to Val-517, Glu-519 to Asp-535.
840780	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 730 as residues: Leu-8 to Gly-14, Pro-151 to Gly-157.
840857	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 731 as residues: Gln-7 to Glu-22, Ala-27 to Arg-46, Ser-138 to Lys-147, Lys-158 to Pro-163, Asn-171 to Glu-187, Glu-202 to Val-208, Glu-234 to Gly-240, Ser-253 to Lys-260, Gln-272 to Pro-279, Arg-292 to Glu-307, Arg-310 to Arg-317, Asp-342 to Gly-351, Pro-367 to Gly-375, Pro-378 to Arg-388, Leu-425 to Ala-447, Arg-536 to Asp-544, Lys-551 to Lys-561, Val-599 to Asp-604, Ser-622 to Ala-630, Pro-653 to Phe-659, Thr-666 to Ile-673, Pro-699 to Phe-705, Asn-709 to Gly-719, Ala-725 to Phe-737.
840862	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 732 as residues: Arg-2 to Pro-12, Lys-32 to Asn-37, His-75 to Asn-82.
840864	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 733 as residues: Pro-17 to Arg-30, Cys-34 to Gly-40, Met-74 to Glu-81, Pro-106 to Asp-111, Val-136 to Cys-147, Asn-192 to Asp-198.
840938	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 735 as residues: Ser-140 to Thr-148, Thr-194 to Lys-202.
841884	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 736 as residues: Thr-34 to Glu-47.
842241	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 737 as residues: Thr-92 to Lys-101, Glu-134 to Thr-142, Glu-149 to Lys-155, Trp-179 to Ser-187, Thr-205 to Arg-211, Ser-218 to Tyr-225, Asp-283 to Gln-290, Glu-292 to Ile-302, Asn-304 to Met-315.
843712	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 738 as residues: Arg-10 to Asn-16, Ala-59 to Pro-67.
844040	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 739 as residues: Phe-59 to Glu-68, Lys-105 to Gly-111.
844617	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 742 as

	residues: Arg-1 to Lys-7.
846187	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 745 as residues: Gly-8 to Gly-14, Gly-41 to Glu-48, Glu-54 to Lys-74, Glu-87 to Arg-98, Thr-158 to Asn-166, Gly-247 to Ser-254, Gly-257 to Arg-277, Ala-437 to Ser-444, Lys-505 to Arg-510, Phe-519 to Tyr-525, Lys-531 to Pro-538, Gly-562 to Leu-571, Phe-606 to Val-613, Val-692 to Ala-697, Ser-705 to Leu-715, Leu-742 to Cys-747.
HANGA53R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 749 as residues: Arg-4 to Ser-9.
HAHCP93R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 752 as residues: Ser-1 to Ser-12, Thr-23 to Arg-28.
HBGAA76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 753 as residues: Ser-4 to Ser-11, Pro-27 to Asn-37.
HTXPI29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 756 as residues: Thr-17 to Leu-24, Thr-57 to Tyr-67, Leu-92 to Phe-102, Asn-128 to Gln-134.
HBGAA54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 760 as residues: Arg-62 to Leu-70, Ile-74 to Arg-79.
HDPJR77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 763 as residues: Glu-7 to Lys-22, Thr-33 to Glu-39, Lys-69 to Glu-76, Asp-84 to Tyr-90.
HTTIO41R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 764 as residues: Val-17 to Ser-22, Arg-41 to Glu-46, Lys-50 to Pro-75, Ser-92 to Pro-100.
HDPUL86R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 767 as residues: Lys-7 to Gly-13.
HTXNT16R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 768 as residues: Leu-67 to Asn-72, Thr-102 to Phe-111, Gly-127 to Gln-135.
HLXNA54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 770 as residues: Gln-1 to Glu-6, Pro-23 to Trp-31, Arg-46 to Trp-51.
H2LAX93R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 772 as residues: Glu-3 to Gln-10.
HWAFW10R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 773 as residues: Glu-13 to Asp-22, His-34 to Trp-40, Arg-69 to Lys-75.
HBGDD17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 775 as residues: Arg-23 to Thr-28, Pro-40 to Glu-51, Ala-62 to His-68.
H2CBB43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 778 as residues: Asp-90 to Asp-95, Arg-106 to Thr-117.
H2CBQ77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 779 as residues: Asp-11 to Gly-16, Gln-19 to Tyr-24, Pro-34 to Gly-46.
HOEMK06R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 781 as residues: Pro-1 to Gln-14.
HCHAG30R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 783 as residues: Gly-1 to Trp-7.
HAEA126R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 788 as residues: Lys-32 to Val-40, Arg-43 to Pro-51.
H2CBN76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 791 as residues: Ala-17 to Leu-22, Thr-72 to Lys-77.
HAGFX49R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 792 as residues: Ala-10 to Leu-15, His-64 to Cys-71.
HTXKR32R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 794 as residues: Ser-2 to Gly-12, Glu-57 to Val-65.
H6EAF46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 796 as residues: Arg-11 to Ser-21.
H2LAK40R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 798 as residues: Glu-11 to Lys-20, Pro-22 to Arg-28.
H2LAY71R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 799 as residues: Arg-26 to Leu-36, Gln-82 to Asp-101, Arg-103 to Arg-108, Arg-113 to Arg-131.
HASAW80R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 803 as

	residues: Gly-1 to Arg-6, Ala-19 to Pro-27, Gly-34 to Phe-40.
HCHAF25R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 804 as residues: Ser-30 to Thr-40, Leu-78 to Val-85, Asp-92 to Ala-97.
HLTHH84R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 805 as residues: Glu-2 to Ala-8.
HADDC09R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 808 as residues: Leu-3 to Gly-9, Thr-20 to Gly-29.
HAQA110R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 811 as residues: Gly-1 to Lys-21.
HBGBT78R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 814 as residues: Asn-1 to Lys-22.
HBGCB06R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 815 as residues: Phe-1 to Phe-15.
HCHMW05R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 823 as residues: Pro-6 to Ser-11.
HODFW25R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 829 as residues: Ser-1 to Thr-8, Glu-17 to Ala-32, Arg-39 to Trp-47.
HOEMQ91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 830 as residues: Arg-8 to Ser-13.
HOGBG56R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 831 as residues: Lys-20 to Arg-25.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or encoded by a polynucleotide that hybridizes to the complement of an epitope encoding sequence of SEQ ID NO:X, or an epitope encoding sequence contained in the deposited cDNA clone under stringent hybridization conditions, or alternatively, under lower stringency hybridization conditions, as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand under stringent hybridization conditions or alternatively, under lower stringency hybridization conditions, as defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at

least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice

are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention, and immunogenic and/or antigenic epitope fragments thereof can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995).

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for



immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).)

5        Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for  
10       convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., *Cell* 37:767 (1984).)

15       Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

20       Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., *Proc. Natl. Acad. Sci. USA* 88:8972- 897 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto  
25       Ni<sup>2+</sup> nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

30       Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al.,

Curr. Opin. Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

As discussed herein, any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In certain preferred embodiments, proteins of the invention comprise fusion proteins wherein the polypeptides are N and/or C- terminal deletion mutants. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions mutants. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

#### 10 **Vectors, Host Cells, and Protein Production**

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include,

but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells.

5 Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-  
10 3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1,  
15 pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in  
20 many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid  
25 extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention can also be recovered from: products purified  
30 from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast,

higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using  $O_2$ . This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for  $O_2$ . Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to

the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the

polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (*see, e.g., Carter et al., Nucl. Acids Res. 13:4331 (1986); and Zoller et al., Nucl. Acids Res. 10:6487 (1982)*), cassette mutagenesis (*see, e.g., Wells et al., Gene 34:315 (1985)*), restriction selection mutagenesis (*see, e.g., Wells et al., Philos. Trans. R. Soc. London SerA 317:415 (1986)*).

The invention additionally, encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased

solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200; 500; 1000; 1500; 2000; 2500; 3000; 3500; 4000; 4500; 5000; 5500; 6000; 6500; 7000; 7500; 8000; 8500; 9000; 9500; 10,000; 10,500; 11,000; 11,500; 12,000; 12,500; 13,000; 13,500; 14,000; 14,500; 15,000; 15,500; 16,000; 16,500; 17,000; 17,500; 18,000; 18,500; 19,000; 19,500; 20,000; 25,000; 30,000; 35,000; 40,000; 50,000; 55,000; 60,000; 65,000; 70,000; 75,000; 80,000; 85,000; 90,000; 95,000; or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a



reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. 5 Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine 10 residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining 15 the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available 20 for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished 30 by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-

304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The breast/ovarian cancer antigen polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present

invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers,  
5 or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or an amino acid sequence encoded by SEQ ID NO:X, and/or an amino acid sequence encoded by the cDNA in a related cDNA clone  
10 contained in a deposited library (including fragments, variants, splice variants, and fusion proteins, corresponding to any one of these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the  
15 invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at  
20 least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric  
25 multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example,  
30 homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention

contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in a polypeptide encoded by SEQ ID NO:X, and/or by the cDNA in the related cDNA clone contained in a deposited library). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the

invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture  
5 supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S.  
10 patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag®  
15 polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention  
20 may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides  
25 desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number  
30 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide

components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

### **Antibodies**

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG,

IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain  
5 Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable  
10 region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human  
15 immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of  
20 a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553  
25 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid  
30 residues. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that

specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention.

In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.



Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon et al., *J. Immunol.* 160(7):3170-3179 (1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol.*

Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to  
5 purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference  
10 herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically  
15 conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the  
20 covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other  
25 protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method  
30 known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to

induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by

fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any

desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869  
5 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al.,  
10 *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant  
15 region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are  
20 antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework  
25 substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.)  
30 Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein*

Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent

No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed  
5 against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903  
10 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies  
15 which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For  
20 example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

#### ***Polynucleotides Encoding Antibodies***

The invention further provides polynucleotides comprising a nucleotide sequence  
25 encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

30 The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be

assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework



regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038-1041 (1988)).

#### ***Methods of Producing Antibodies***

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not

limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or

factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells.

- 5 The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

- In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence  
10 of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan &  
15 Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can  
20 be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

- In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion  
25 desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end,  
30 eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS,

MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or ap<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215; and hyg<sup>r</sup>, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.),

Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by  
5 reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing  
10 antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the  
15 first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to  
20 avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method  
25 known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide  
30 sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or

portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using

methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995)).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent



materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical

chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, 5 International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.* 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), 10 granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not 15 limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 20 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of 25 Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an 30 antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

## 5 ***Immunophenotyping***

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types.

10 Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S.

15 Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic

20 stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

## ***Assays For Antibody Binding***

The antibodies of the invention may be assayed for immunospecific binding by any

25 method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays,

30 immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel *et al.*, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York,

which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., <sup>3</sup>H or <sup>125</sup>I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., <sup>3</sup>H or <sup>125</sup>I) in the presence of increasing amounts of an unlabeled second antibody.

## ***Therapeutic Uses***

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of

the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities

include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

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### *Gene Therapy*

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989).

In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the



host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method

known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, *Cell* 71:973-985 (1992); Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); and Pittelkow and Scott, *Mayo Clinic Proc.* 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that

expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### 15 *Therapeutic/Prophylactic Administration and Composition*

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral

routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al.,

J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

5 Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector  
10 and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868  
15 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means  
20 approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic  
25 origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate,  
30 glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form

of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend

on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

5 For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life  
10 foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more  
15 containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

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### ***Diagnosis and Imaging***

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity  
25 of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide  
30 gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the



amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99m}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule

is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### *Kits*

5           The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present  
10           invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes  
15           the first antibody may be conjugated to a detectable substrate).

          In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated  
20           polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically  
25           synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

          In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of  
30           the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

#### **Uses of the Polynucleotides**

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The breast/ovarian cancer antigen polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X, or the complement thereto. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 3 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation  
5 hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000);  
10 and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular  
15 disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

20 Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all  
25 affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

30 Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the

invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention provides a method of detecting increased or decreased expression levels of the breast, ovarian, breast cancer and/or ovarian cancer polynucleotides in affected individuals as compared to unaffected individuals using polynucleotides of the present invention and techniques known in the art, including but not limited to the method described in Example 11. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder related to the female reproductive system, particularly a disorder related to the breast and/or ovary, including breast cancer and/or ovarian cancer, involving measuring the expression level of breast/ovarian cancer antigen polynucleotides in breast and/or ovarian tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard breast, ovarian, breast cancer and/or ovarian cancer polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder related to the female reproductive system, particularly a disorder related to the breast and/or ovary, including breast cancer and/or ovarian cancer.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'-mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder related to the female reproductive system, particularly a disorder related to the breast and/or ovary, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed breast, ovarian, breast cancer and/or ovarian cancer polynucleotide expression will experience a

worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of breast, ovarian, breast cancer and/or ovarian cancer polynucleotides" is intended qualitatively or quantitatively measuring or estimating the level of the breast, ovarian, breast cancer and/or ovarian cancer polypeptide or the level of the mRNA encoding the breast, ovarian, breast cancer and/or ovarian cancer polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the breast, ovarian, breast cancer and/or ovarian cancer polypeptide level or mRNA level in a second biological sample). Preferably, the breast, ovarian, breast cancer and/or ovarian cancer polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard breast, ovarian, breast cancer and/or ovarian cancer polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the female reproductive system related disorder or being determined by averaging levels from a population of individuals not having a female reproductive system related disorder. As will be appreciated in the art, once a standard breast, ovarian, breast cancer and/or ovarian cancer polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains breast, ovarian, breast cancer and/or ovarian cancer polypeptide or the corresponding mRNA. As indicated, biological samples include body fluids (such as vaginal pool, breast milk, lymph, sera, plasma, urine, semen, synovial fluid and spinal fluid) which contain the breast, ovarian, breast cancer and/or ovarian cancer polypeptide, breast and/or ovarian tissue, and other tissue sources found to express the breast, ovarian, breast cancer and/or ovarian cancer polypeptide. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with breast, ovarian, breast cancer and/or ovarian cancer polynucleotides attached may

be used to identify polymorphisms between the breast, ovarian, breast cancer and/or ovarian cancer polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions, though most preferably in breast and/or ovarian related proliferative, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses breast, ovarian, breast cancer and/or ovarian cancer polynucleotides that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T<sub>sub.m</sub>) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.



The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

10 Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in *Neoplastic Diseases of the Blood*, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification  
15 of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in some  
20 animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580).  
25 However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., *Proc. Natl. Acad. Sci.* 85:1028 (1988); Anfossi et al., *Proc.*  
30 *Natl. Acad. Sci.* 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not limited to treatment of proliferative disorders of hematopoietic

cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a breast/ovarian cancer antigen polynucleotide can be used to control gene expression through triple helix formation or through antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed

on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

5       The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA  
10       sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues,  
15       e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific  
20       polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular  
25       tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to breast, ovarian, breast cancer and/or ovarian cancer polynucleotides prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue  
30       cultures for contamination.

The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample.

Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower  
5 levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention, breast, ovarian, breast cancer and/or ovarian cancer tissues and/or cancerous and/or wounded tissues) or bodily fluids (e.g., vaginal pool, breast milk, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder,  
10 relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in  
15 the assayed gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the  
20 process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### **Uses of the Polypeptides**

25 Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J.  
30 Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for

5 detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Ti}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ;

10 luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying levels of polypeptide of the present invention in a biological

15 sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be

20 incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ , ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Ti}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ),

25 molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ,  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system

30 used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or

antibody fragment will then preferentially accumulate at the location of cells which express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ , or other radioisotopes such as, for example,  $^{103}\text{Pd}$ ,  $^{133}\text{Xe}$ ,  $^{131}\text{I}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ ,  $^{90}\text{Yttrium}$ ,  $^{117}\text{Tin}$ ,  $^{186}\text{Rhenium}$ ,  $^{166}\text{Holmium}$ , and  $^{188}\text{Rhenium}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; 5 and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a breast, ovarian, breast cancer and/or ovarian cancer polypeptide of the present invention in cells or body fluid of an individual, or more  
10 preferably, assaying the expression level of a breast, ovarian, breast cancer and/or ovarian cancer of the present invention in breast and/or ovarian cells or vaginal pool or breast milk of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With  
15 respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or  
20 further progression of the cancer.

Moreover, breast/ovarian cancer antigen polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or  
25 cancerous diseases and conditions, preferably proliferative disorders of the breast and/or ovary, and/or cancerous disease and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the  
30 activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing

inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

#### **Gene Therapy Methods**

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Belldgrun, A., et al., *J. Natl. Cancer Inst.* 85: 207-216 (1993); Ferrantini, M. et al., *Cancer Research* 53: 1107-1112 (1993); Ferrantini, M. et al., *J. Immunology* 153: 4604-4615 (1994); Kaido, T., et al., *Int. J. Cancer* 60: 221-229 (1995); Ogura, H., et al., *Cancer Research* 50: 5102-5106 (1990); Santodonato, L., et al., *Human Gene Therapy* 7:1-10 (1996);



Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-

actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into  
5 cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis,  
10 ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the  
15 lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example,  
20 stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more  
25 preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

30 The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues,

throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical  
5 administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

10 In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have  
15 been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

20 Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include  
25 transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes  
30 is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG),  
5 dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can  
10 be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat  
15 Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

20 The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by  
25 depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate  
30 solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to

the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., *Proc. Natl. Acad. Sci. USA* (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., *Proc. Natl. Acad. Sci. USA* (1978) 75:145; Schaefer-Ridder et al., *Science* (1982) 215:166), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy* 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector

may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and  $\text{CaPO}_4$  precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

5       The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express a polypeptide of the present invention.

10       In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have  
15       been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis. 109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive  
20       studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

      Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al.,  
25       Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to  
30       Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc.

Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, including direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

Preferably, the polynucleotide encoding a polypeptide of the present invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the



cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such

carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

#### 15 **Biological Activities**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

#### **Immune Activity**

A polypeptide or polynucleotide, or agonists or antagonists of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides or polypeptides, or

agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells.

5 Polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g.  
10 agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, DiGeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

15 Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia,  
20 factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

25 Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides or polypeptides, or agonists or  
30 antagonists of the present invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

**Hyperproliferative Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may  
5 inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing  
10 T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by  
15 Polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

20 Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease,  
25 histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

30 Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403

(1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral  
5 (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-  
10 dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time  
15 of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells.  
20 The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.  
25

The present invention is further directed to antibody-based therapies which involve  
30 administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal

antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998),



which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., *Cancer Metastasis Rev.* 17(2):155-61 (1998), which is hereby incorporated by reference)).

5 Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-  
10 mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., *Eur J Biochem* 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the  
15 activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, *Mutat Res* 400(1-2):447-55 (1998), *Med Hypotheses*.50(5):423-33 (1998), *Chem Biol Interact.* Apr 24;111-112:23-34 (1998), *J Mol Med.*76(6):402-12 (1998), *Int J Tissue React*;20(1):3-15 (1998), which are all hereby incorporated by reference).

20 Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., *Curr Top*  
25 *Microbiol Immunol* 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or  
30 polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with

heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

#### 10 **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilog  
15 of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, heart murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomas, bacillary angiomas, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, ataxia telangiectasia, hereditary hemorrhagic telangiectasia, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a

Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

#### Anti-Angiogenesis Activity

5           The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and  
10           spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye  
15           disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological  
20           conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

          The polynucleotides encoding a polypeptide of the present invention may be administered along with other polynucleotides encoding an angiogenic protein. Examples of  
25           angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide  
30           synthase.

          The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the

invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including breast, ovarian, prostate, lung, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid

arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque  
5 neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar  
10 or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of  
15 hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental  
20 fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular  
25 degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalm.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalm.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating  
30 neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation

of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired



potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

5           Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma.

10          Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a  
15          therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

            Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist  
20          in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

            Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such  
25          that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

            Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic  
30          joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated

with anti-angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-

chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolimidazole; and metalloproteinase inhibitors such as BB94.

## 5 Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung  
10 cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary  
15 cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression,  
20 and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute  
25 lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not  
30 limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's

tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

#### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity

wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentopial graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on

the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associated with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or



polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

#### **Neurological Diseases**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as

cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis, cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache, migraine, dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, Hallervorden-Spatz Syndrome, hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous

system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, cerebral malaria, meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis. Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis, Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uve-meningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie) cerebral toxoplasmosis, central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral scleriosis which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucopolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as

holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta, hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary  
5 optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing  
10 loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering,  
15 voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus,  
20 Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color  
25 vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease, muscular atrophy such as  
30 spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex

Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, Diabetic neuropathies such as diabetic foot, nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

#### **Infectious Disease**

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, 5 Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, 10 Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, 15 Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of 20 the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially 25 available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram- 30 Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia

(e.g., *Borrelia burgdorferi*, Brucellosis, Candidiasis, *Campylobacter*, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacteriaceae (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*,  
 5 Legionellosis, Leptospirosis, *Listeria*, Mycoplasmatales, *Mycobacterium leprae*, *Vibrio cholerae*, Neisseriaceae (e.g., *Acinetobacter*, Gonorrhea, Meningococcal), *Meisseria meningitidis*, Pasteurellaceae Infections (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza type B*), *Pasteurella*), *Pseudomonas*, Rickettsiaceae, Chlamydiaceae, Syphilis, *Shigella* spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g.,  
 10 *Streptococcus pneumoniae* and Group B *Streptococcus*). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme  
 15 Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections.  
 20 Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, Ppolynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated or  
 25 detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and *Trichomonas* and Sporozoans (e.g., *Plasmodium virax*, *Plasmodium falciparum*,  
 30 *Plasmodium malariae* and *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic

infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### **Regeneration**

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.



Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

#### **Chemotaxis**

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

#### **Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules  
5 include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide  
10 binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the  
15 expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled  
20 competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound  
25 with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the  
30 polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand

panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a  
5 cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

10 Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be  
15 photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide  
20 probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S.  
25 Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and  
30 corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding

polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and  $^3\text{[H]}$  thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of  $^3\text{[H]}$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $^3\text{[H]}$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the

present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

### **Targeted Delivery**

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method

for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

5 In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector  
10 systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an  
15 inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be  
20 used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

## 25 Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of  
30 having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the

polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells  
5 which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other  
10 agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is  
15 separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and  
20 is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known  
25 in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention  
30 specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

**Antisense And Ribozyme (Antagonists)**

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained in the cDNA contained in the related cDNA clone identified in Table 1. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J., *Neurochem.* 56:560 (1991). *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., *Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the



production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invention or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980)), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most

efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of polynucleotide sequences described herein could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3'- or coding region of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre et al., 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,

2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 5 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, 10 and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or 15 analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric 20 RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides 25 may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

30 Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at

site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'.

5 The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA; i.e., to increase efficiency and minimize the  
10 intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding  
15 DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

20 Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and  
25 prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

30 The antagonist/agonist may also be employed to treat the diseases described herein.

Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with

overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

## 5 **Other Activities**

A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The  
10 polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells  
15 and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's  
20 disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be  
25 also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and  
30 differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

#### **Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least  
5 50 contiguous nucleotides is included in the nucleotide sequence of an open reading frame sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the  
10 deposit.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

15 A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a  
20 sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and  
25 determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected  
30 from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a



nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleotide sequence of SEQ ID NO:X; or the cDNA in the related cDNA clone identified in Table 1 which encodes a protein, wherein the method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence of the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for diagnosing a pathological condition which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the

group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

5 Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a DNA microarray or "chip" of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 500, 1000, 2000, 3000 or 4000 nucleotide sequences, wherein at least one sequence in said DNA microarray or "chip" is at least 95% identical to a sequence of at least 50 contiguous  
10 nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the cDNA clone referenced in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least  
15 90% identical to a sequence of at least about 10 contiguous amino acids in the polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid  
20 sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a  
25 polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

30 Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a portion of said polypeptide encoded by the cDNA clone referenced in Table 1; a polypeptide encoded by SEQ ID NO:X; and/or the polypeptide sequence of SEQ ID NO:Y.

5 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid  
10 sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide  
15 comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is a method for detecting in a biological sample a polypeptide  
20 comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule  
25 in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from  
30 said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in

a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleic acid sequence identified in Table 1 encoding a polypeptide, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of:

polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a  
5 prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

10 Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

15 Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a human protein comprising an amino acid sequence selected from the group consisting of:  
20 polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such an individual a  
25 Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

Also preferred is a method of treatment of an individual in need of a decreased level of a protein activity, which method comprised administering to such an individual a  
30 Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to decrease the level of said protein activity in said individual.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

*Examples**Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample*

5 Each deposited cDNA clone is contained in a plasmid vector. Table 5 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The following correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 5 as being isolated in the vector "Lambda Zap,"  
 10 the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
15	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
20	pCR <sup>®</sup> 2.1	pCR <sup>®</sup> 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are  
 25 commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3  
 30

primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 5, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited by reference to Table 2 and 5 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone referenced in Table 1.



### TABLE 5

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HUKA HUKB HUKC HUKD HUKF HUKF HUKG	Human Uterine Cancer	Lambda ZAP II	LP01
HICNA HCNB	Human Colon	Lambda Zap II	LP01
HFFA	Human Fetal Brain, random primed	Lambda Zap II	LP01
HTWA	Resting T-Cell	Lambda ZAP II	LP01
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP01
HLMB HLMF HLMG HLMH HLMI HLMJ HLMN HLMN	breast lymph node CDNA library	Lambda ZAP II	LP01
HCQA HCQB	human colon cancer	Lamda ZAP II	LP01
HMEA HMEC HMED HMEE HMEF HMEG HMEI HMEJ HMEK HMEI	Human Microvascular Endothelial Cells, fract. A	Lambda ZAP II	LP01
HUSA HUSC	Human Umbilical Vein Endothelial Cells, fract. A	Lambda ZAP II	LP01
HLQA HLQB	Hepatocellular Tumor	Lambda ZAP II	LP01
HHGA HHGB HHGC HHGD	Hemangiopericytoma	Lambda ZAP II	LP01
HSDM	Human Striatum Depression, re-rescue	Lambda ZAP II	LP01
HUSH	H Umbilical Vein Endothelial Cells, fract. A, re-excision	Lambda ZAP II	LP01
HSGS	Salivary gland, subtracted	Lambda ZAP II	LP01
HFXA HFXB HFXC HFXD HFXE HFXF HFXG HFXH	Brain frontal cortex	Lambda ZAP II	LP01
HPQA HPQB HPQC	PERM TF274	Lambda ZAP II	LP01
HFXJ HFXK	Brain Frontal Cortex, re-excision	Lambda ZAP II	LP01
HICWA HCWB HCWC HCWD HCWE HCWF HCWG HCWH HCWI HCWJ HCWK	CD34 positive cells (Cord Blood)	ZAP Express	LP02
HCUA HCUB HCUC	CD34 depleted Buffy Coat (Cord Blood)	ZAP Express	LP02
HIRSM	A-14 cell line	ZAP Express	LP02
HRSA	A1-CELL LINE	ZAP Express	LP02
HICUD HCUE HCUF HCUG HCUH HCUI	CD34 depleted Buffy Coat (Cord Blood), re-excision	ZAP Express	LP02
HBXE HBXF HBXG	H. Whole Brain #2, re-excision	ZAP Express	LP02
HRLM	L8 cell line	ZAP Express	LP02
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP02
HUDA HUDB HUDC	Testes	ZAP Express	LP02
HHTM HHTN HHTO	H. hypothalamus, frac A;re-excision	ZAP Express	LP02
HHTL	H. hypothalamus, frac A	ZAP Express	LP02
HASA HASD	Human Adult Spleen	Uni-ZAP XR	LP03
HFKC HFKD HFKE HFKF HFKG	Human Fetal Kidney	Uni-ZAP XR	LP03
HE8A HE8B HE8C HE8D HE8E HE8F HE8M HE8N	Human 8 Week Whole Embryo	Uni-ZAP XR	LP03
HGBA HGBD HGBE HGBF HGBG HGBH HGBI	Human Gall Bladder	Uni-ZAP XR	LP03
HLHA HLHB HLHC HLHD HLHE HLHF HLHG HLHH HLHQ	Human Fetal Lung III	Uni-ZAP XR	LP03
HPMA HPMB HPMC HPMD HPME HPMF HPMG HPMH	Human Placenta	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP03
HSIA HSIC HSID HSIE	Human Adult Small Intestine	Uni-ZAP XR	LP03
HTEA HTEB HTEC HTED HTEE HTEF HTEG HTEH HTEI HTEJ HTEK	Human Testes	Uni-ZAP XR	LP03
HTPA HTPB HTPC HTPD HTPF	Human Pancreas Tumor	Uni-ZAP XR	LP03
HTTA HTTB HTTC HTTD HTTE HTTF	Human Testes Tumor	Uni-ZAP XR	LP03
HAPA HAPB HAPC HAPM	Human Adult Pulmonary	Uni-ZAP XR	LP03
HETA HETB HETC HETD HETE HETF HETG HETI HETJ HETK	Human Endometrial Tumor	Uni-ZAP XR	LP03
HHFB HHFC HHFD HHFE HHFF HHFG HHFH HHFI	Human Fetal Heart	Uni-ZAP XR	LP03
HHPB HHPD HHPF HHPG HHPH	Human Hippocampus	Uni-ZAP XR	LP03
HCE1 HCE2 HCE3 HCE4 HCE5 HCEB HCEC HCE4 HCEE HCEF HCEG	Human Cerebellum	Uni-ZAP XR	LP03
HUVB HUVC HUVD HUVE	Human Umbilical Vein. Endo. remake	Uni-ZAP XR	LP03
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP03
HTAA HTAB HTAC HTAD HTAE	Human Activated T-Cells	Uni-ZAP XR	LP03
HFEA HFEB HFEC	Human Fetal Epithelium (Skin)	Uni-ZAP XR	LP03
HJPA HJPB HJPC HJPD	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	Uni-ZAP XR	LP03
HESA	Human epithelioid sarcoma	Uni-Zap XR	LP03
HLTA HLTB HLTC HLTD HLTE HLTF	Human T-Cell Lymphoma	Uni-ZAP XR	LP03
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP03
HRDA HRDB HRDC HRDD HRDE HRDF	Human Rhabdomyosarcoma	Uni-ZAP XR	LP03
HCAA HCAB HCAC	Cem cells cyclohexamide treated	Uni-ZAP XR	LP03
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HSUA HSUB HSUC HSUM	Supt Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HT4A HT4C HT4D	Activated T-Cells, 12 hrs.	Uni-ZAP XR	LP03
HE9A HE9B HE9C HE9D HE9E HE9F HE9G HE9H HE9M HE9N	Nine Week Old Early Stage Human	Uni-ZAP XR	LP03
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP03
HT5A	Activated T-Cells, 24 hrs.	Uni-ZAP XR	LP03
HFGA HFGM	Human Fetal Brain	Uni-ZAP XR	LP03
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP03
HBGB HBGD	Human Primary Breast Cancer	Uni-ZAP XR	LP03
HBNA HBNB	Human Normal Breast	Uni-ZAP XR	LP03
HCAS	Cem Cells, cyclohexamide treated, subtra	Uni-ZAP XR	LP03
HHPS	Human Hippocampus. subtracted	pBS	LP03
HKCS HKCU	Human Colon Cancer. subtracted	pBS	LP03
HRGS	Raji cells, cyclohexamide treated, subtracted	pBS	LP03
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBS	LP03
HT4S	Activated T-Cells, 12 hrs, subtracted	Uni-ZAP XR	LP03
HCDA HCDB HCDC HCDD HCDE	Human Chondrosarcoma	Uni-ZAP XR	LP03
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP03
HTLA HTLB HTLC HTLD HTLE	Human adult testis. large inserts	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HTLF			
HLMA HLMC HLMD	Breast Lymph node cDNA library	Uni-ZAP XR	LP03
H6EA H6EB H6EC	HL-60, PMA 4H	Uni-ZAP XR	LP03
HTXA HTXB HTXC HTXD HTXE HTXF HTXG HTXH	Activated T-Cell (12hs)/Thiouridine labelledEco	Uni-ZAP XR	LP03
HNFA HNFH HNFC HNFD HNFE HNFF HNFG HNFH HNFJ	Human Neutrophil, Activated	Uni-ZAP XR	LP03
HTOB HTOC	HUMAN TONSILS, FRACTION 2	Uni-ZAP XR	LP03
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP03
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP03
HORB	Human OB HOS treated (10 nM E2) fraction I	Uni-ZAP XR	LP03
HSVA HSVB HSVC	Human Chronic Synovitis	Uni-ZAP XR	LP03
HROA	HUMAN STOMACH	Uni-ZAP XR	LP03
HBJA HBJB HBJC HBJD HBJE HBJF HBJG HBJH HBJI HBJJ HBJK	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP03
HCRA HCRB HCRC	human corpus colosum	Uni-ZAP XR	LP03
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP03
HDSA	Dermatofibrosarcoma Protuberance	Uni-ZAP XR	LP03
HMWA HMWB HMWC HMWD HMWE HMWF HMWG HMWH HMWI HMWJ	Bone Marrow Cell Line (RS4:11)	Uni-ZAP XR	LP03
HSOA	stomach cancer (human)	Uni-ZAP XR	LP03
HERA	SKIN	Uni-ZAP XR	LP03
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP03
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP03
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP03
HBCA HBCB	H. Lymph node breast Cancer	Uni-ZAP XR	LP03
HPWT	Human Prostate BPH, re-excision	Uni-ZAP XR	LP03
HFVG HFVH HFVI	Fetal Liver, subtraction II	pBS	LP03
HNFI	Human Neutrophils, Activated, re- excision	pBS	LP03
HBMB HBMC HBMD	Human Bone Marrow, re-excision	pBS	LP03
HKML HKMM HKMN	H. Kidney Medulla, re-excision	pBS	LP03
HKIX HKIY	H. Kidney Cortex, subtracted	pBS	LP03
HADT	H. Amygdala Depression, subtracted	pBS	LP03
H6AS	HL-60, untreated, subtracted	Uni-ZAP XR	LP03
H6ES	HL-60, PMA 4H, subtracted	Uni-ZAP XR	LP03
H6BS	HL-60, RA 4h, Subtracted	Uni-ZAP XR	LP03
H6CS	HL-60, PMA 1d, subtracted	Uni-ZAP XR	LP03
HTXJ HTXK	Activated T-cell(12h)/Thiouridine-re- excision	Uni-ZAP XR	LP03
HMSA HMSB HMSC HMSD HMSE HMSF HMSG HMSH HMSI HMSJ HMSK	Monocyte activated	Uni-ZAP XR	LP03
HAGA HAGB HAGC HAGD HAGE HAGF	Human Amygdala	Uni-ZAP XR	LP03
HSRA HSRB HSRE	STROMAL -OSTEOCLASTOMA	Uni-ZAP XR	LP03
HSRD HSRF HSRG HSRH	Human Osteoclastoma Stromal Cells - unamplified	Uni-ZAP XR	LP03
HSQA HSQB HSQC HSQD HSQE	Stromal cell TF274	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HSQF HSQG			
HSKA HSKB HSKC HSKD HSKE HSKF HSKZ	Smooth muscle, serum treated	Uni-ZAP XR	LP03
HSLA HSLB HSLC HSLD HSLE HSLF HSLG	Smooth muscle, control	Uni-ZAP XR	LP03
HSDA HSDD HSDE HSDF HSDG HSDH	Spinal cord	Uni-ZAP XR	LP03
HPWS	Prostate-BPH subtracted II	pBS	LP03
HSKW HSKX HSKY	Smooth Muscle- HASTE normalized	pBS	LP03
HFPB HFPC HFPD	H. Frontal cortex, epileptic, re-excision	Uni-ZAP XR	LP03
HSDI HSDJ HSDK	Spinal Cord, re-excision	Uni-ZAP XR	LP03
HSKN HSKO	Smooth Muscle Serum Treated, Norm	pBS	LP03
HSKG HSKH HSKI	Smooth muscle, serum induced, re-exc	pBS	LP03
HFCA HFCB HFCC HFCD HFCE HFCF	Human Fetal Brain	Uni-ZAP XR	LP04
HPTA HPTB HPTD	Human Pituitary	Uni-ZAP XR	LP04
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP04
HE6B HE6C HE6D HE6E HE6F HE6G HE6S	Human Whole Six Week Old Embryo	Uni-ZAP XR	LP04
HSSA HSSB HSSC HSSD HSSE HSSF HSSG HSSH HSSI HSSJ HSSK HE7T	Human Synovial Sarcoma 7 Week Old Early Stage Human, subtracted	Uni-ZAP XR	LP04
HEPA HEPB HEPC	Human Epididymus	Uni-ZAP XR	LP04
HSNA HSNB HSNC HSNM HSNN	Human Synovium	Uni-ZAP XR	LP04
HPFB HPFC HPFD HPFE	Human Prostate Cancer, Stage C fraction	Uni-ZAP XR	LP04
HE2A HE2D HE2E HE2H HE2I HE2M HE2N HE2O	12 Week Old Early Stage Human	Uni-ZAP XR	LP04
HE2B HE2C HE2F HE2G HE2P HE2Q	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP04
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP04
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP04
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP04
HWTA HWTB HWTC	wilm's tumor	Uni-ZAP XR	LP04
HBSD	Bone Cancer, re-excision	Uni-ZAP XR	LP04
HSGB	Salivary gland, re-excision	Uni-ZAP XR	LP04
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP04
HSXA HSXB HSXC HSXD	Human Substantia Nigra	Uni-ZAP XR	LP04
HSIA HSHB HSHC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP04
HOUA HOUB HOUH HOUD HOUE	Adipocytes	Uni-ZAP XR	LP04
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP04
HELA HELB HELC HELD HELE HELF HELG HELH	Endothelial cells-control	Uni-ZAP XR	LP04
HEMA HEMB HEMC HEMD HEME HEMF HEMG HEMH	Endothelial-induced	Uni-ZAP XR	LP04
HBIA HBIB HBIC	Human Brain, Striatum	Uni-ZAP XR	LP04
HHSA HHSB HHSC HHSD HHSE	Human Hypothalamus, Schizophrenia	Uni-ZAP XR	LP04
HNGA HNGB HNGC HNGD HNGE HNGF HNGG HNGH HNGI HNGJ	neutrophils control	Uni-ZAP XR	LP04
HNHA HNHB HNHC HNHD HNHE HNHF HNIG HNHH HNHI HNHI	Neutrophils IL-1 and LPS induced	Uni-ZAP XR	LP04
HSDB HSDC	STRIATUM DEPRESSION	Uni-ZAP XR	LP04

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HHPT	Hypothalamus	Uni-ZAP XR	LP04
HSAT HSAU HSAV HSAW HSAX HSAY HSAZ	Anergic T-cell	Uni-ZAP XR	LP04
HBMS HBMT HBMU HBMV HBMW HBMX	Bone marrow	Uni-ZAP XR	LP04
HOEA HOEB HOEC HOED HOEE HOEF HOEJ	Osteoblasts	Uni-ZAP XR	LP04
HAIA HAIB HAIC HAID HAIE HAIF	Epithelial-TNF $\alpha$ and INF induced	Uni-ZAP XR	LP04
HTGA HTGB HTGC HTGD	Apoptotic T-cell	Uni-ZAP XR	LP04
HMCA HMCB HMCC HMCD HMCE	Macrophage-oxLDL	Uni-ZAP XR	LP04
HMAA HMAB HMAB HMAD HMAE HMAF HMAG	Macrophage (GM-CSF treated)	Uni-ZAP XR	LP04
HPHA	Normal Prostate	Uni-ZAP XR	LP04
HPIA HPIB HPIC	LNCAP prostate cell line	Uni-ZAP XR	LP04
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP04
HOSE HOSF HOSG	Human Osteoclastoma, re-excision	Uni-ZAP XR	LP04
HTGE HTGF	Apoptotic T-cell, re-excision	Uni-ZAP XR	LP04
HMAJ HMAK	H Macrophage (GM-CSF treated), re-excision	Uni-ZAP XR	LP04
HACB HACC HADC	Human Adipose Tissue, re-excision	Uni-ZAP XR	LP04
HFPA	H. Frontal Cortex, Epileptic	Uni-ZAP XR	LP04
HFAA HFAB HFAC HFAD HFAE	Alzheimers, spongy change	Uni-ZAP XR	LP04
HFAM	Frontal Lobe, Dementia	Uni-ZAP XR	LP04
HMIA HMIB HMIC	Human Manic Depression Tissue	Uni-ZAP XR	LP04
HTSA HTSE HTSF HTSG HTSH	Human Thymus	pBS	LP05
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBS	LP05
HSAA HSAB HSAC	HSA 172 Cells	pBS	LP05
HSBA HSBB HSBC HSBM	HSC172 cells	pBS	LP05
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBS	LP05
HJBA HJBB HJBC HJBD	Jurkat T-Cell, S phase	pBS	LP05
HAFA HAFB	Aorta endothelial cells + TNF- $\alpha$	pBS	LP05
HAWA HAWB HAWC	Human White Adipose	pBS	LP05
HTNA HTNB	Human Thyroid	pBS	LP05
HONA	Normal Ovary, Premenopausal	pBS	LP05
HARA HARB	Human Adult Retina	pBS	LP05
HLJA HLJB	Human Lung	pCMVSPORT 1	LP06
HOFM HOFN HOFQ	H. Ovarian Tumor, II, OV5232	pCMVSPORT 2.0	LP07
HOGA HOGB HOGC	OV 10-3-95	pCMVSPORT 2.0	LP07
HCGL	CD34+cells, II	pCMVSPORT 2.0	LP07
HDLA	Hodgkin's Lymphoma I	pCMVSPORT 2.0	LP07
HDTA HDTB HDTC HDTD HDTE	Hodgkin's Lymphoma II	pCMVSPORT 2.0	LP07
HKAA HKAB HKAC HKAD HKAE HKAF HKAG HKAH	Keratinocyte	pCMVSPORT2.0	LP07
HCIM	CAPFINDER, Crohn's Disease, lib 2	pCMVSPORT 2.0	LP07
HKAL	Keratinocyte, lib 2	pCMVSPORT2.0	LP07
HKAT	Keratinocyte, lib 3	pCMVSPORT2.0	LP07
HNDA	Nasal polyps	pCMVSPORT2.0	LP07
HDRA	H. Primary Dendritic Cells, lib 3	pCMVSPORT2.0	LP07

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HOHA HOHB HOHC	Human Osteoblasts II	pCMVSPORT2.0	LP07
HLDA HLDB HLDC	Liver, Hepatoma	pCMVSPORT3.0	LP08
HLDN HLDO HLDP	Human Liver, normal	pCMVSPORT3.0	LP08
HMTA	pBMC stimulated w/ poly I/C	pCMVSPORT3.0	LP08
HNTA	NTERA2, control	pCMVSPORT3.0	LP08
HDBA HDBB HDBC HDBD HDBE HDBF HDBG HDBH HDBI HDBJ HDBK HDBL HDBM HDBN HDBO HDBP	Primary Dendritic Cells, lib 1	pCMVSPORT3.0	LP08
HDBM HDBN HDBO HDBP	Primary Dendritic cells, frac 2	pCMVSPORT3.0	LP08
HMUA HMUB HMUC	Myeloid Progenitor Cell Line	pCMVSPORT3.0	LP08
HHEA HHEB HHEC HHED	T Cell helper I	pCMVSPORT3.0	LP08
HHEM HHEN HHEO HHEP	T cell helper II	pCMVSPORT3.0	LP08
HEQA HEQB HEQC	Human endometrial stromal cells	pCMVSPORT3.0	LP08
HJMA HJMB	Human endometrial stromal cells-treated with progesterone	pCMVSPORT3.0	LP08
HSWA HSWB HSWC	Human endometrial stromal cells-treated with estradiol	pCMVSPORT3.0	LP08
HSYA HSYB HSYC	Human Thymus Stromal Cells	pCMVSPORT3.0	LP08
HLWA HLWB HLWC	Human Placenta	pCMVSPORT3.0	LP08
HRAA HRAB HRAC	Rejected Kidney, lib 4	pCMVSPORT3.0	LP08
HMTM	PCR, pBMC I/C treated	PCR II	LP09
HMJA	H. Meningioma, M6	pSport 1	LP10
HMKA HMKB HMKC HMKD HMKE	H. Meningioma, M1	pSport 1	LP10
HUSG HUSI	Human umbilical vein endothelial cells, IL-4 induced	pSport 1	LP10
HUSX HUSY	Human Umbilical Vein Endothelial Cells, uninduced	pSport 1	LP10
HOFA	Ovarian Tumor 1, OV5232	pSport 1	LP10
HCFA HCFB HCFC HCFD	T-Cell PHA 16 hrs	pSport 1	LP10
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport 1	LP10
HADA HADC HADD HADE HADF HADG	Human Adipose	pSport 1	LP10
HOVA HOVB HOVC	Human Ovary	pSport 1	LP10
HTWB HTWC HTWD HTWE HTWF	Resting T-Cell Library, II	pSport 1	LP10
HIMA	Spleen metastatic melanoma	pSport 1	LP10
HLYA HLYB HLYC HLYD HLYE	Spleen, Chronic lymphocytic leukemia	pSport 1	LP10
HCGA	CD34+ cell, I	pSport 1	LP10
HEOM HEON	Human Eosinophils	pSport 1	LP10
HTDA	Human Tonsil, Lib 3	pSport 1	LP10
HSPA	Salivary Gland, Lib 2	pSport 1	LP10
HCHA HCHB HCHC	Breast Cancer cell line, MDA 36	pSport 1	LP10
HCHM HCHN	Breast Cancer Cell line, angiogenic	pSport 1	LP10
HCIA	Crohn's Disease	pSport 1	LP10
HDAA HDAB HDAC	HEL cell line	pSport 1	LP10
HABA	Human Astrocyte	pSport 1	LP10
HUFA HUFB HUF C	Ulcerative Colitis	pSport 1	LP10
HNTM	NTERA2 + retinoic acid, 14 days	pSport 1	LP10
HDQA	Primary Dendritic cells, CapFinder2, frac 1	pSport 1	LP10
HDQM	Primary Dendritic Cells, CapFinder, frac	pSport 1	LP10

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	2		
HLDX	Human Liver, normal.CapFinder	pSport 1	LP10
HULA HULB HULC	Human Dermal Endothelial Cells,untreated	pSport1	LP10
HUMA	Human Dermal Endothelial cells,treated	pSport1	LP10
HCJA	Human Stromal Endometrial fibroblasts, untreated	pSport1	LP10
HCJM	Human Stromal endometrial fibroblasts, treated w/ estradiol	pSport1	LP10
HEDA	Human Stromal endometrial fibroblasts, treated with progesterone	pSport1	LP10
HFNA	Human ovary tumor cell OV350721	pSport1	LP10
HKGA HKGB HKGC HKGD	Merkel Cells	pSport1	LP10
HISA HISB HISC	Pancreas Islet Cell Tumor	pSport1	LP10
HLSA	Skin, burned	pSport1	LP10
HIBZA	Prostate.BPH, Lib 2	pSport 1	LP10
HBZS	Prostate BPH.Lib 2, subtracted	pSport 1	LP10
HFIA HFIB HFIC	Synovial Fibroblasts (control)	pSport 1	LP10
HFIH HFII HFII	Synovial hypoxia	pSport 1	LP10
HFIT HFIU HFIV	Synovial IL-1/TNF stimulated	pSport 1	LP10
HGCA	Mesangial cell, frac 1	pSport1	LP10
HMVA HMVB HMVC	Bone Marrow Stromal Cell, untreated	pSport1	LP10
HFIX HFII HFIZ	Synovial Fibroblasts (IL1/TNF), sub	pSport1	LP10
HFOX HFOY HFOZ	Synovial hypoxia-RSF subtracted	pSport1	LP10
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP11
HLIA HLIB HLIC	Human Liver	pCMVSPORT 1	LP012
HHBA HHBB HHBC HHBD HHBE	Human Heart	pCMVSPORT 1	LP012
HBBA HBBB	Human Brain	pCMVSPORT 1	LP012
HLJA HLJB HLJC HLJD HLJE	Human Lung	pCMVSPORT 1	LP012
HOGA HOGB HOGC	Ovarian Tumor	pCMVSPORT 2.0	LP012
HTJM	Human Tonsils, Lib 2	pCMVSPORT 2.0	LP012
HAMF HAMG	KMH2	pCMVSPORT 3.0	LP012
HAJA HAJB HAJC	L428	pCMVSPORT 3.0	LP012
HWBA HWBB HWBC HWBD HWBE	Dendritic cells, pooled	pCMVSPORT 3.0	LP012
HWAA HWAB HWAC HWAD HWAE	Human Bone Marrow, treated	pCMVSPORT 3.0	LP012
HYAA HYAB HYAC	B Cell lymphoma	pCMVSPORT 3.0	LP012
HWHG HWHH HWHI	Healing groin wound, 6.5 hours post incision	pCMVSPORT 3.0	LP012
HWHP HWHQ HWHR	Healing groin wound; 7.5 hours post incision	pCMVSPORT 3.0	LP012
HARM	Healing groin wound - zero hr post-incision (control)	pCMVSPORT 3.0	LP012
HBIM	Olfactory epithelium; nasalcavity	pCMVSPORT 3.0	LP012
HWDA	Healing Abdomen wound; 70&90 min post incision	pCMVSPORT 3.0	LP012
HWEA	Healing Abdomen Wound;15 days post incision	pCMVSPORT 3.0	LP012
HWJA	Healing Abdomen Wound;21&29 days	pCMVSPORT 3.0	LP012
HNAL	Human Tongue, frac 2	pSport1	LP012
HMJA	H. Meningima, M6	pSport1	LP012
HMKA HMKB HMKC HMKE	H. Meningima, M1	pSport1	LP012

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HOFA	Ovarian Tumor I, OVS232	pSport I	LP012
HCFA HCFB HCFC HCFC	T-Cell PHA 16 hrs	pSport I	LP012
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport I	LP012
HMMA HMMB HMMC	Spleen metastatic melanoma	pSport I	LP012
HTDA	Human Tonsil, Lib 3	pSport I	LP012
HDBA	Human Fetal Thymus	pSport I	LP012
HIDUA	Pericardium	pSport I	LP012
HBZA	Prostate.BPH, Lib 2	pSport I	LP012
HWCA	Larynx tumor	pSport I	LP012
HWKA	Normal lung	pSport I	LP012
HSMB	Bone marrow stroma,treated	pSport I	LP012
HBHM	Normal trachea	pSport I	LP012
HLFC	Human Larynx	pSport I	LP012
HLRB	Siebben Polyposis	pSport I	LP012
HNIA	Mammary Gland	pSport I	LP012
HNJB	Palate carcinoma	pSport I	LP012
HNKA	Palate normal	pSport I	LP012
HMZA	Pharynx carcinoma	pSport I	LP012
HABG	Cheek Carcinoma	pSport I	LP012
HMZM	Pharynx Carcinoma	pSport I	LP012
HDRM	Larynx Carcinoma	pSport I	LP012
HVAA	Pancreas normal PCA4 No	pSport I	LP012
HICA	Tongue carcinoma	pSport I	LP012
HUKA HUKB HUKC HUKD HUKF	Human Uterine Cancer	Lambda ZAP II	LP013
HFFA	Human Fetal Brain, random primed	Lambda ZAP II	LP013
HTUA	Activated T-cell labeled with 4-thioluri	Lambda ZAP II	LP013
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP013
HMEB	Human microvascular Endothelial cells, fract. B	Lambda ZAP II	LP013
HIUSH	Human Umbilical Vein Endothelial cells, fract. A, re-excision	Lambda ZAP II	LP013
HLQC HLQD	Hepatocellular tumor, re-excision	Lambda ZAP II	LP013
HTWJ HTWK HTWL	Resting T-cell, re-excision	Lambda ZAP II	LP013
HF6S	Human Whole 6 week Old Embryo (II), subt	pBluescript	LP013
HHPS	Human Hippocampus, subtracted	pBluescript	LP013
HLIS	LNCAP, differential expression	pBluescript	LP013
HLHS HLHT	Early Stage Human Lung, Subtracted	pBluescript	LP013
HSUS	Supt cells, cyclohexamide treated, subtracted	pBluescript	LP013
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBluescript	LP013
HSDS	H. Striatum Depression, subtracted	pBluescript	LP013
HPTZ	Human Pituitary, Subtracted VII	pBluescript	LP013
HSDX	H. Striatum Depression, subt II	pBluescript	LP013
HSDZ	H. Striatum Depression, subt	pBluescript	LP013
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBluescript SK-	LP013
HRTA	Colorectal Tumor	pBluescript SK-	LP013
HISBA HSBB HSBC HSBM	HSC172 cells	pBluescript SK-	LP013
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBluescript SK-	LP013
HJBA HJBB HJBC HJBD	Jurkat T-cell, S1 phase	pBluescript SK-	LP013



Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HTNA HTNB	Human Thyroid	pBluescript SK-	LP013
HAHA HAHB	Human Adult Heart	Uni-ZAP XR	LP013
HE6A	Whole 6 week Old Embryo	Uni-ZAP XR	LP013
HFCA HFCE HFCC HFCD HFCE	Human Fetal Brain	Uni-ZAP XR	LP013
HFKE HFKE HFKE HFKE HFKE	Human Fetal Kidney	Uni-ZAP XR	LP013
HGBA HGBD HGBE HGBF HGBG	Human Gall Bladder	Uni-ZAP XR	LP013
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP013
HTEA HTEB HTEC HTEH HTEE	Human Testes	Uni-ZAP XR	LP013
HTTA HTTB HTTC HTTD HTEE	Human Testes Tumor	Uni-ZAP XR	LP013
HYBA HYBB	Human Fetal Bone	Uni-ZAP XR	LP013
HFLA	Human Fetal Liver	Uni-ZAP XR	LP013
HHFB HHFC HHFD HHFE HHFF	Human Fetal Heart	Uni-ZAP XR	LP013
HUVB HUVB HUVB HUVB HUVB	Human Umbilical Vein, End. remake	Uni-ZAP XR	LP013
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP013
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP013
HTAA HTAB HTAC HTAD HTAE	Human Activated T-cells	Uni-ZAP XR	LP013
HFEA HFEB HFEC	Human Fetal Epithelium (skin)	Uni-ZAP XR	LP013
HJPA HJPB HJPC HJPD	Human Jurkat Membrane Bound Polysomes	Uni-ZAP XR	LP013
HESA	Human Epithelioid Sarcoma	Uni-ZAP XR	LP013
HALS	Human Adult Liver, Subtracted	Uni-ZAP XR	LP013
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP013
HCAA HCAB HCAC	Cem cells, cyclohexamide treated	Uni-ZAP XR	LP013
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP013
HE9A HE9B HE9C HE9D HE9E	Nine Week Old Early Stage Human	Uni-ZAP XR	LP013
HSFA	Human Fibrosarcoma	Uni-ZAP XR	LP013
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP013
HTRA	Human Trachea Tumor	Uni-ZAP XR	LP013
HE2A HE2D HE2E HE2H HE2I	12 Week Old Early Stage Human	Uni-ZAP XR	LP013
HE2B HE2C HE2F HE2G HE2P	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP013
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP013
HBGA	Human Primary Breast Cancer	Uni-ZAP XR	LP013
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP013
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP013
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP013
HTOA HTOD HTOE HTOF HTOG	human tonsils	Uni-ZAP XR	LP013
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP013
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP013
HOQB	Human OB HOS treated (1 nM E2) fraction I	Uni-ZAP XR	LP013
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP013
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP013
HROA HROC	HUMAN STOMACH	Uni-ZAP XR	LP013
HBJA HBJB HBJC HBJD HBJE	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP013
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP013
HCPA	Corpus Callosum	Uni-ZAP XR	LP013
HSEA	stomach cancer (human)	Uni-ZAP XR	LP013
HERA	SKIN	Uni-ZAP XR	LP013
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP013
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HWTA HWTB HWTC	Wilms tumor	Uni-ZAP XR	LP013
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP013
HAPN HAPO HAPP HAPQ HAPR	Human Adult Pulmonary; re-excision	Uni-ZAP XR	LP013
HLTG HLTH	Human T-cell lymphoma; re-excision	Uni-ZAP XR	LP013
HAHC HAHD HAHE	Human Adult Heart; re-excision	Uni-ZAP XR	LP013
HAGA HAGB HAGC HAGD HAGE	Human Amygdala	Uni-ZAP XR	LP013
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP013
HSHA HSHB HSHC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP013
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP013
HP1A HP1B HP1C	LNCAP prostate cell line	Uni-ZAP XR	LP013
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP013
HBTA	Bone Marrow Stroma. TNF&LPS ind	Uni-ZAP XR	LP013
HMCF HMCB HMCH HMCJ HMCJ	Macrophage-oxLDL; re-excision	Uni-ZAP XR	LP013
HAGG HAGH HAGI	Human Amygdala; re-excision	Uni-ZAP XR	LP013
HACA	H. Adipose Tissue	Uni-ZAP XR	LP013
HKFB	K562 + PMA (36 hrs); re-excision	ZAP Express	LP013
HCWT HCWU HCWV	CD34 positive cells (cord blood); re-ex	ZAP Express	LP013
HBWA	Whole brain	ZAP Express	LP013
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP013
HAVM	Temporal cortex-Alzheimer	pT-Adv	LP014
HAVT	Hippocampus, Alzheimer Subtracted	pT-Adv	LP014
HHAS	CHME Cell Line	Uni-ZAP XR	LP014
HAJR	Larynx normal	pSport 1	LP014
HWLE HWLF HWLG HWLH	Colon Normal	pSport 1	LP014
HCRM HCRN HCRO	Colon Carcinoma	pSport 1	LP014
HWLI HWLJ HWLK	Colon Normal	pSport 1	LP014
HWLQ HWLR HWLS HWLT	Colon Tumor	pSport 1	LP014
HBFM	Gastrocnemius Muscle	pSport 1	LP014
HBOD HBOE	Quadriceps Muscle	pSport 1	LP014
HBKD HBKE	Soleus Muscle	pSport 1	LP014
HCCM	Pancreatic Langerhans	pSport 1	LP014
HWGA	Larynx carcinoma	pSport 1	LP014
HWGM HWGN	Larynx carcinoma	pSport 1	LP014
HWLA HWLB HWLC	Normal colon	pSport 1	LP014
HWLM HWLN	Colon Tumor	pSport 1	LP014
HVAM HVAN HVAO	Pancreas Tumor	pSport 1	LP014
HWGQ	Larynx carcinoma	pSport 1	LP014
HAQM HAQN	Salivary Gland	pSport 1	LP014
HASM	Stomach; normal	pSport 1	LP014
HBCM	Uterus; normal	pSport 1	LP014
HCDM	Testis; normal	pSport 1	LP014
HDJM	Brain; normal	pSport 1	LP014
HEFM	Adrenal Gland; normal	pSport 1	LP014
HBAA	Rectum normal	pSport 1	LP014
HFDH	Rectum tumour	pSport 1	LP014
HGAM	Colon; normal	pSport 1	LP014
HHMM	Colon; tumour	pSport 1	LP014
HCLB HCLC	Human Lung Cancer	Lambda Zap II	LP015
HRLA	L1 Cell line	ZAP Express	LP015

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HHAM	Hypothalamus, Alzheimer's	pCMVSPORT 3.0	LP015
HKBA	Ku 812F Basophils Line	pSport 1	LP015
HS2S	Saos2, Dexamethosone Treated	pSport 1	LP016
HA5A	Lung Carcinoma A549 TNFalpha activated	pSport 1	LP016
HTFM	TF-1 Cell Line GM-CSF Treated	pSport 1	LP016
HYAS	Thyroid Tumour	pSport 1	LP016
HUTS	Larynx Normal	pSport 1	LP016
HXOA	Larynx Tumor	pSport 1	LP016
HEAII	Ea.hy 926 cell line	pSport 1	LP016
HINA	Adenocarcinoma Human	pSport 1	LP016
HRMA	Lung Mesothelium	pSport 1	LP016
HLCL	Human Pre-Differentiated Adipocytes	Uni-Zap XR	LP017
HS2A	Saos2 Cells	pSport 1	LP020
HS2I	Saos2 Cells, Vitamin D3 Treated	pSport 1	LP020
HUCM	CHME Cell Line, untreated	pSport 1	LP020
HEPN	Aryepiglottis Normal	pSport 1	LP020
HPSN	Sinus Piniiformis Tumour	pSport 1	LP020
HNSA	Stomach Normal	pSport 1	LP020
HNSM	Stomach Tumour	pSport 1	LP020
HNLA	Liver Normal Met5No	pSport 1	LP020
HUTA	Liver Tumour Met 5 Tu	pSport 1	LP020
HOCN	Colon Normal	pSport 1	LP020
HOCT	Colon Tumor	pSport 1	LP020
HTNT	Tongue Tumour	pSport 1	LP020
HLXN	Larynx Normal	pSport 1	LP020
HLXT	Larynx Tumour	pSport 1	LP020
HTYN	Thymus	pSport 1	LP020
HPLN	Placenta	pSport 1	LP020
HTNG	Tongue Normal	pSport 1	LP020
HZAA	Thyroid Normal (SDCA2 No)	pSport 1	LP020
HWES	Thyroid Thyroiditis	pSport 1	LP020
HFHD	Ficollled Human Stromal Cells, 5Fu treated	pTriplEx2	LP021
HFHM,HFHN	Ficollled Human Stromal Cells, Untreated	pTriplEx2	LP021
HPCI	Hep G2 Cells, lambda library	lambda Zap-CMV XR	LP021
HBCA,HBCB,HBCC	H. Lymph node breast Cancer	Uni-ZAP XR	LP021
HCOK	Chondrocytes	pSPORT1	LP022
HDCA, HDCB, HDCC	Dendritic Cells From CD34 Cells	pSPORT1	LP022
HDMA, HDMB	CD40 activated monocyte dendritic cells	pSPORT1	LP022
HDDM, HDDN, HDDO	LPS activated derived dendritic cells	pSPORT1	LP022
HPCR	Hep G2 Cells, PCR library	lambda Zap-CMV XR	LP022
HAAA, HAAB, HAAC	Lung, Cancer (4005313A3): Invasive Poorly Differentiated Lung Adenocarcinoma	pSPORT1	LP022
HIPA, HIPB, HIPC	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic	pSPORT1	LP022
HOOH, HOOI	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low	pSPORT1	LP022

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	Malignant Pot		
HIDA	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HUJA.HUJB.HUJC.HUJD.HUJE	B-Cells	pCMVSPORT 3.0	LP022
HNOA.HNOB.HNOC.HNOD	Ovary, Normal: (9805C040R)	pSPORT1	LP022
HNLN	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HSCL	Stromal Cells	pSPORT1	LP022
HAAX	Lung, Cancer: (4005313 A3) Invasive Poorly-differentiated Metastatic lung adenocarcinoma	pSPORT1	LP022
HUUA.HUUB.HUUC.HUUD	B-cells (unstimulated)	pTriplEx2	LP022
HWWA.HWWB.HWWC.HWWD.HWWE.HWWF.HWWG	B-cells (stimulated)	pSPORT1	LP022
HCCC	Colon, Cancer: (9808C064R)	pCMVSPORT 3.0	LP023
HPDO HPDP HPDQ HPDR HPD	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	pSport 1	LP023
HPCO HPCP HPCQ HPCT	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	pSport 1	LP023
HOCM HOCO HOCQ HOCQ	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	pSport 1	LP023
HCBM HCBN HCBO	Breast, Cancer: (4004943 A5)	pSport 1	LP023
HNBH HNBV HNBV	Breast, Normal: (4005522B2)	pSport 1	LP023
HBCP HBCQ	Breast, Cancer: (4005522 A2)	pSport 1	LP023
HBCJ	Breast, Cancer: (9806C012R)	pSport 1	LP023
HSAM HSAN	Stromal cells 3.88	pSport 1	LP023
HVCA HVCB HVCC HVCD	Ovary, Cancer: (4004332 A2)	pSport 1	LP023
HSCK HSEN HSEO	Stromal cells (HBM3.18)	pSport 1	LP023
HSCP HSCQ	stromal cell clone 2.5	pSport 1	LP023
HUXA	Breast Cancer: (4005385 A2)	pSport 1	LP023
HCOM HCON HCOO HCOP HCOQ	Ovary, Cancer (4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	pSport 1	LP023
HBNM	Breast, Cancer: (9802C020E)	pSport 1	LP023
HVVA HVVB HVVC HVVD HVVE	Human Bone Marrow, treated	pSport 1	LP023

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 5. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to the nucleotide sequence of SEQ ID NO:X.

5 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid  
10 mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using  
15 Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the  
20 nucleotide sequence of SEQ ID NO:X are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu\text{l}$  of reaction mixture with 0.5  $\mu\text{g}$  of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin, 20  $\mu\text{M}$  each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of  
25 Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA  
30 product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not

limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

5 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full  
10 length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the  
15 RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis  
20 using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

25

### ***Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide***

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the sequence corresponding to SEQ ID NO:X, according to the method  
30 described in Example 1. (See also, Sambrook.)

***Example 3: Tissue specific expression analysis***

The Human Genome Sciences, Inc. (HGS) database is derived from sequencing tissue specific cDNA libraries. Libraries generated from a particular tissue are selected and the specific tissue expression pattern of EST groups or assembled contigs within these libraries is determined by comparison of the expression patterns of those groups or contigs within the entire database. ESTs which show tissue specific expression are selected.

The original clone from which the specific EST sequence was generated, is obtained from the catalogued library of clones and the insert amplified by PCR using methods known in the art. The PCR product is denatured then transferred in 96 well format to a nylon membrane (Schleicher and Scheull) generating an array filter of tissue specific clones. Housekeeping genes, maize genes, and known tissue specific genes are included on the filters. These targets can be used in signal normalization and to validate assay sensitivity. Additional targets are included to monitor probe length and specificity of hybridization.

Radioactively labeled hybridization probes are generated by first strand cDNA synthesis per the manufacturer's instructions (Life Technologies) from mRNA/RNA samples prepared from the specific tissue being analyzed. The hybridization probes are purified by gel exclusion chromatography, quantitated, and hybridized with the array filters in hybridization bottles at 65°C overnight. The filters are washed under stringent conditions and signals are captured using a Fuji phosphorimager.

Data is extracted using AIS software and following background subtraction, signal normalization is performed. This includes a normalization of filter-wide expression levels between different experimental runs. Genes that are differentially expressed in the tissue of interest are identified and the full length sequence of these clones is generated.

***Example 4: Chromosomal Mapping of the Polynucleotides***

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute

cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR  
5 fragment in the particular somatic cell hybrid.

***Example 5: Bacterial Expression of a Polypeptide***

A polynucleotide encoding a polypeptide of the present invention is amplified using  
10 PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial  
15 expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is  
20 ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is  
25 isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto  
30 pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.



Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction

sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express  
5 protein in a bacterial system.

***Example 6: Purification of a Polypeptide from an Inclusion Body***

The following alternative method can be used to purify a polypeptide expressed in *E*  
10 *coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell  
15 paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then  
20 mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is  
25 discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous  
30 stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16  $\mu$ m membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu$ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### *Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System*

25

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under

control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

5 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

10 Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon, is amplified using the PCR protocol described in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard  
15 methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with  
20 appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

25 The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the  
30 cloned fragment is confirmed by DNA sequencing.

Five  $\mu$ g of a plasmid containing the polynucleotide is co-transfected with 1.0  $\mu$ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA",

Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One  $\mu\text{g}$  of BaculoGold™ virus DNA and 5  $\mu\text{g}$  of the plasmid are mixed in a sterile well of a microtiter plate containing 50  $\mu\text{l}$  of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10  $\mu\text{l}$  Lipofectin plus 90  $\mu\text{l}$  Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu\text{l}$  of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu\text{Ci}$  of  $^{35}\text{S}$ -methionine and 5  $\mu\text{Ci}$   $^{35}\text{S}$ -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

*Example 8: Expression of a Polypeptide in Mammalian Cells*

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used

for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 or pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10,

25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### 10 *Example 9: Protein Fusions*

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

25 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the



vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

5 If the naturally occurring signal sequence is used to produce the polypeptide of the present invention, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

10 GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCACCGTGCCCAG  
CACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGA  
CACCCTCATGATCTCCCGGACTCCTGAGGTACATGCGTGGTGGTGGACGTAAGC  
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT  
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC  
15 AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC  
AAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGAAAACCATCTCCAAGCC  
AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAG  
CTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC  
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGAC  
20 CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC  
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT  
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT  
GAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:837)

25 *Example 10: Production of an Antibody from a Polypeptide*

#### **a) Hybridoma Technology**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing  
30 polypeptide of the present invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide

of the present invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide of the present invention are prepared  
5 using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide of the present invention or, more preferably, with a secreted polypeptide of the present invention-  
10 expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell  
15 line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a  
20 selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide of the present invention.

Alternatively, additional antibodies capable of binding to polypeptide of the present invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is  
25 possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide of the present invention-specific antibody can be blocked by polypeptide of the  
30 present invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide of the present invention-specific antibody and are used to immunize an animal to induce formation of further polypeptide of the present invention-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

**b) Isolation Of Antibody Fragments Directed Against Polypeptide of the Present Invention From A Library Of scFvs**

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

*Rescue of the Library.* A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10<sup>9</sup> E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra

8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10<sup>13</sup> transducing units/ml (ampicillin-resistant clones).

*Panning of the Library.* Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10<sup>13</sup> TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

*Characterization of Binders.* Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

*Example 11: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide*

RNA isolated from entire families or individual patients presenting with a phenotype  
5 of interest (such as a disease) is be isolated. cDNA is then generated from these RNA  
samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a  
template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X;  
and/or the nucleotide sequence of the related cDNA in the cDNA clone contained in a  
deposited library. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30  
10 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using  
buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4  
polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The  
intron-exon borders of selected exons is also determined and genomic PCR products  
15 analyzed to confirm the results. PCR products harboring suspected mutations is then cloned  
and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic  
Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States  
Biochemical). Affected individuals are identified by mutations not present in unaffected  
20 individuals.

Genomic rearrangements are also observed as a method of determining alterations in  
a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2  
are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim),  
and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991).  
25 Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA  
for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium  
iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are  
obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination  
with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable  
30 excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image

collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

*Example 12: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample*

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

*Example 13: Formulation*

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed  
5 herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good  
10 medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

15 As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given  
20 continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

25 Therapeutics can be are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of  
30 administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray.

5 "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

10 Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

15 Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

20 Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci.(USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being  
30 adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987);



Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated  
5 generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the  
10 Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such  
15 carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate,  
20 succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including  
25 cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be  
30 understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is

readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

5           Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic  
10       using bacteriostatic Water-for-Injection.

          The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products,  
15       which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

          The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention  
20       include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include,  
25       but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping  
30       cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or

concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate  
5 administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories,  
10 conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous  
15 lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta),  
20 LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7  
25 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or

prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone,

azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

10 In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, 25 perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, 30 interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine);

cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2

(VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO  
5 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in  
10 combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be  
15 administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation  
20 therapy.

*Example 14: Method of Treating Decreased Levels of the Polypeptide*

The present invention relates to a method for treating an individual in need of an  
25 increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a polypeptide of the present invention in an individual can be treated by administering the  
30 agonist or antagonist of the present invention. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the agonist or



antagonist to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the agonist or antagonist for six consecutive days. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 13.

5

*Example 15: Method of Treating Increased Levels of the Polypeptide*

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to  
10 such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, due to a variety of etiologies, such as cancer.

15 For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 13.

20 *Example 16: Method of Treatment Using Gene Therapy-Ex Vivo*

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces.  
25 Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated  
30 at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer

is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on  
5 agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a  
10 HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the  
15 gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce  
20 infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer  
25 cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his.  
30 Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after

having been grown to confluence on cytodex 3 microcarrier beads.

*Example 17: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention*

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Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; 10 International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting 15 sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct 20 restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the 25 appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

30 In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral

particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub>HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately  $3 \times 10^6$  cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V,

respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and  
5 the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having  
10 been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

*Example 18: Method of Treatment Using Gene Therapy - In Vivo*

15 Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present  
20 invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al.,  
25 Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

30 The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell,

including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will

appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

#### *Example 19: Transgenic Animals*

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (*i.e.*, polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, *e.g.*, Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campbell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci.



USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

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*Example 20: Knock-Out Animals*

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*, animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding

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sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

*Example 22: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation*

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a

positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

**In Vitro Assay-** Agonists or antagonists of the invention can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the agonists or antagonists of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well)

with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

**In Vivo Assay-** BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of agonists or antagonists of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with agonists or antagonists of the invention identify the results of the activity of the agonists or antagonists on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with agonist or antagonist is used to indicate whether the agonists or antagonists specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice. Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and agonists or antagonists-treated mice.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

#### *Example 23: T Cell Proliferation Assay*

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of <sup>3</sup>H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10<sup>4</sup>/well) of mAb coated plates

in RPMI containing 10% FCS and P/S in the presence of varying concentrations of agonists or antagonists of the invention (total volume 200  $\mu$ l). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100  $\mu$ l of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100  $\mu$ l of medium containing 0.5 uCi of  $^3$ H-thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3$ H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of agonists or antagonists of the invention.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 24: Effect of Agonists or Antagonists of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells*

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC $\gamma$ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of agonist or antagonist of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow

cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly  
5 influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6$ /ml) are treated with increasing concentrations of agonists or antagonists of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using  
10 commercial ELISA kit (e.g, R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules,  
15 molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

20 FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of agonists or antagonists of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are  
25 analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine  
30 whether a molecule of the invention functions as an inhibitor or activator of monocytes. Agonists or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified

from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

- 5 Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in  
10 polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in  
15 this experimental paradigm.

- Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows.  
20 Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing concentrations of agonists or antagonists of the invention and under the same conditions, but in the absence of agonists or antagonists. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of agonist or antagonist of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use.  
25 Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e. g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

- Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing  
30 concentrations of agonists or antagonists of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To



the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The  
5 absorbance is read at 610 nm. To calculate the amount of H<sub>2</sub>O<sub>2</sub> produced by the macrophages, a standard curve of a H<sub>2</sub>O<sub>2</sub> solution of known molarity is performed for each experiment.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test  
10 the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 25: Biological Effects of Agonists or Antagonists of the Invention*

15 Astrocyte and Neuronal Assays.

Agonists or antagonists of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for  
20 the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate an agonist or antagonist of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on  
25 cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not  
30 necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal

culture paradigm, the ability of an agonist or antagonist of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

5    Fibroblast and endothelial cell assays.

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate  
10    for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays,  
15    the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or agonists or antagonists of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one  
20    day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without agonists or antagonists of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or agonists or antagonists of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on  
25    growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with agonists or antagonists of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of  
30    striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection

neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in  
5 dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has  
10 trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, agonists or antagonists of the invention can be  
15 evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of an agonist or antagonist of the invention is first examined *in vitro* in a  
20 dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with  
25 paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a  
30 developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*.

Therefore, if an agonist or antagonist of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the agonist or antagonist may be involved in Parkinson's Disease.

5 The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

10 *Example 26: The Effect of Agonists or Antagonists of the Invention on the Growth of Vascular Endothelial Cells*

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2.5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. An agonist or antagonist of the invention, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

20 An increase in the number of HUVEC cells indicates that the compound of the invention may proliferate vascular endothelial cells, while a decrease in the number of HUVEC cell indicates that the compound of the invention inhibits vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

25

*Example 27: Rat Corneal Wound Healing Model*

This animal model shows the effect of an agonist or antagonist of the invention on neovascularization. The experimental protocol includes:

- 30 a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- b) Inserting a spatula below the lip of the incision facing the outer corner of the

eye.

c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).

d) Positioning a pellet, containing 50ng- 5ug of an agonist or antagonist of the invention, within the pocket.

5 e) Treatment with an agonist or antagonist of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test  
10 the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 28: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models*

*A. Diabetic db+/db+ Mouse Model.*

15 To demonstrate that an agonist or antagonist of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner,  
20 M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal  
25 recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-  
30 55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*,

*Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

5           The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

          Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The  
10       animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

15           Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical  
20       area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with  
25       sterile saline and gauze sponges.

          Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no  
30       longer visible and the wound is covered by a continuous epithelium.

          An agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups

received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with an agonist or antagonist of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue

control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

- 5           Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

#### *B. Steroid Impaired Rat Model*

- The inhibition of wound healing by steroids has been well documented in various *in vitro* and  
10 *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *Am. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*,  
15 *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish  
20 phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

- To demonstrate that an agonist or antagonist of the invention can accelerate the  
25 healing process, the effects of multiple topical applications of the agonist or antagonist on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

- Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are  
30 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All



manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of  
5 wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The  
10 wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on  
15 days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups  
20 received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

25 Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the  
30 differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned  
5 perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine  
hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds.  
Histologic examination of the wounds allows assessment of whether the healing process and  
the morphologic appearance of the repaired skin is improved by treatment with an agonist or  
antagonist of the invention. A calibrated lens micrometer is used by a blinded observer to  
10 determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is  
considered significant.

The studies described in this example tested activity of agonists or antagonists of the  
invention. However, one skilled in the art could easily modify the exemplified studies to test  
15 the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

#### *Example 29: Lymphadema Animal Model*

The purpose of this experimental approach is to create an appropriate and consistent  
20 lymphedema model for testing the therapeutic effects of an agonist or antagonist of the  
invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in  
the rat hind limb. Effectiveness is measured by swelling volume of the affected limb,  
quantification of the amount of lymphatic vasculature, total blood plasma protein, and  
histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly,  
25 the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis.  
Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the  
right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in  
70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric  
30 measurements are made prior to injecting dye into paws after marking 2 measurement levels  
(0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left  
paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric

measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

**Circumference Measurements:** Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

**Volumetric Measurements:** On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped

into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca<sup>2+</sup> comparison.

5       Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

10       Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test  
15       the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 30: Suppression of TNF alpha-induced adhesion molecule expression by a Agonist or Antagonist of the Invention*

20       The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial  
25       leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

30       Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of an agonist or antagonist of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

- 5 To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or  
10 until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

- Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well  
15 plate to confluence. Growth medium is removed from the cells and replaced with 90  $\mu$ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added  
20 to each well. Plates are held at 4°C for 30 min.

- Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1  
25 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

- Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH  
30 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate

wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPN reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on  
5 blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test  
10 the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 31: Production Of Polypeptide of the Invention For High-Throughput Screening Assays*

15 The following protocol produces a supernatant containing polypeptide of the present invention to be tested. This supernatant can then be used in the Screening Assays described in Examples 33-42.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working  
20 solution of 50ug/ml. Add 200  $\mu$ l of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for  
25 up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

30 The next day, mix together in a sterile solution basin: 300  $\mu$ l Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing

a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add  
5 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then  
10 person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with  
15 1x penstrep, or HGS CHO-5 media (116.6 mg/L of CaCl<sub>2</sub> (anhyd); 0.00130 mg/L CuSO<sub>4</sub>-5H<sub>2</sub>O; 0.050 mg/L of Fe(NO<sub>3</sub>)<sub>3</sub>-9H<sub>2</sub>O; 0.417 mg/L of FeSO<sub>4</sub>-7H<sub>2</sub>O; 311.80 mg/L of Kcl; 28.64 mg/L of MgCl<sub>2</sub>; 48.84 mg/L of MgSO<sub>4</sub>; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO<sub>3</sub>; 62.50 mg/L of NaH<sub>2</sub>PO<sub>4</sub>-H<sub>2</sub>O; 71.02 mg/L of Na<sub>2</sub>HPO<sub>4</sub>; .4320 mg/L of ZnSO<sub>4</sub>-7H<sub>2</sub>O; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-  
20 Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L- Arginine-HCL; 7.50 mg/ml of L-Asparagine-H<sub>2</sub>O; 6.65 mg/ml of L-Aspartic Acid; 29.56  
25 mg/ml of L-Cystine-2HCL-H<sub>2</sub>O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L- Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L- Histidine-HCL-H<sub>2</sub>O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22  
30 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H<sub>2</sub>O; and 99.65 mg/ml of L-

Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 33-40.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide of the present invention directly (e.g., as a secreted protein) or by polypeptide of the present invention inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### *Example 32: Construction of GAS Reporter Construct*

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements



alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:838)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

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			<u>JAKs</u>		<u>STATS GAS(elements) or ISRE</u>	
	<u>Ligand</u>	<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	
	<u>IFN family</u>					
5	IFN-a/B	+	+	-	-	1,2,3 ISRE
	IFN-g		+	+	-	1 GAS (IRF1>Lys6>IFP)
	Il-10	+	?	?	-	1,3
	<u>gp130 family</u>					
10	IL-6 (Pleiotrohic)	+	+	+	?	1,3 GAS (IRF1>Lys6>IFP)
	Il-11(Pleiotrohic)	?	+	?	?	1,3
	OnM(Pleiotrohic)	?	+	+	?	1,3
	LIF(Pleiotrohic)	?	+	+	?	1,3
	CNTF(Pleiotrohic)	-/+	+	+	?	1,3
15	G-CSF(Pleiotrohic)	?	+	?	?	1,3
	IL-12(Pleiotrohic)	+	-	+	+	1,3
	<u>g-C family</u>					
	IL-2 (lymphocytes)	-	+	-	+	1,3,5 GAS
20	IL-4 (lymph/myeloid)	-	+	-	+	6 GAS (IRF1 = IFP
	>>Ly6)(IgH)					
	IL-7 (lymphocytes)	-	+	-	+	5 GAS
	IL-9 (lymphocytes)	-	+	-	+	5 GAS
	IL-13 (lymphocyte)	-	+	?	?	6 GAS
25	IL-15	?	+	?	+	5 GAS
	<u>gp140 family</u>					
	IL-3 (myeloid)	-	-	+	-	5 GAS (IRF1>IFP>>Ly6)
	IL-5 (myeloid)	-	-	+	-	5 GAS
30	GM-CSF (myeloid)	-	-	+	-	5 GAS
	<u>Growth hormone family</u>					
	GH	?	-	+	-	5
	PRL	?	+/-	+	-	1,3,5
35	EPO	?	-	+	-	5 GAS(B-

CAS&gt;IRF1=IFP&gt;&gt;Ly6)

Receptor Tyrosine Kinases

	EGF	?	+	+	-	1,3	GAS (IRF1)
5	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 33-34, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

10 5':GCGCCTCGAGATTTCCTCGAAATCTAGATTTCCTCGAAATGATTTCCTCG  
GAAATGATTTCCTCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:839)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:840)

15 PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

20 5':CTCGAGATTTCCTCGAAATCTAGATTTCCTCGAAATGATTTCCTCGAAA  
TGATTTCCTCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCG  
CCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCT  
CCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCC  
TCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTA  
25 GGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:841)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using Sall and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 33-34.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 35 and 36. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

*Example 33: High-Throughput Screening Assay for T-cell Activity.*

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the

GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC  
5 Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately  
10 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells  
15 containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

20 During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

25 The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptide of the present invention or polypeptide of the present invention induced polypeptides as produced by the protocol described in Example 31.

30 On the day of treatment with the supernatant, the cells should be washed and

resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

5           Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

          After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12  
10   channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

          The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul  
15   samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 37. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

20           As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

          The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

25

*Example 34: High-Throughput Screening Assay Identifying Myeloid Activity*

          The following protocol is used to assess myeloid activity of polypeptide of the present invention by determining whether polypeptide of the present invention  
30   proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using

the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

5 To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 32, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml  
10 penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675 uM  $\text{CaCl}_2$ . Incubate at 37 degrees C for 45 min.

15 Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

20 These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example  
25 31. Incubate at 37 degree C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 37.

30 *Example 35: High-Throughput Screening Assay Identifying Neuronal Activity.*



When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by polypeptide of the present invention.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by polypeptide of the present invention can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:842)

5' GCCAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:843)

Using the GAS:SEAP/Neo vector produced in Example 32, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and

allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and  
5 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 31. EGR-SEAP/PC12 stable cells are obtained by  
10 growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS  
15 (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$   
20 cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 31, 37 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over  
25 fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 37.

*Example 36: High-Throughput Screening Assay for T-cell Activity*

30 NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide

variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 31. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:844), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC  
TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:845)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:840)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCC  
ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCTAACTCCGCCC  
5 ATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA  
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA  
TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA  
GCTT:3' (SEQ ID NO:846)

Next, replace the SV40 minimal promoter element present in the pSEAP2-  
10 promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and  
HindIII. However, this vector does not contain a neomycin resistance gene, and  
therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP  
cassette is removed from the above NF-KB/SEAP vector using restriction enzymes  
15 SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly,  
the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the  
GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are  
created and maintained according to the protocol described in Example 33. Similarly,  
20 the method for assaying supernatants with these stable Jurkat T-cells is also described  
in Example 33. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to  
wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### *Example 37: Assay for SEAP Activity*

25

As a reporter molecule for the assays described in Examples 33-36, SEAP  
activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the  
following general procedure. The Tropix Phospho-light Kit supplies the Dilution,  
Assay, and Reaction Buffers used below.

30

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x

dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### Reaction Buffer Formulation:

15

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25

24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

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*Example 38: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability*

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants  
5 which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to  
10 measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star  
15 black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate  
20 is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2-5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension.  
25 The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

30 For a non-cell based assay, each well contains a fluorescent molecule, such as

fluo-4 . The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either polypeptide of the present invention or a molecule induced by polypeptide of the present invention, which has resulted in an increase in the intracellular  $\text{Ca}^{++}$  concentration.

10

*Example 40: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity*

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether polypeptide of the present invention or a molecule induced by polypeptide of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol

30



is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 31, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4

degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

5           Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for  
10 a range of tyrosine kinases and are available from Boehringer Mannheim.

          The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>,  
15 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

          The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

20           Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-  
25 POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

          Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound  
30 peroxidase activity is quantitated using an ELISA reader and reflects the level of

tyrosine kinase activity.

*Example 41: High-Throughput Screening Assay Identifying Phosphorylation Activity*

5           As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 40, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other  
10   molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

          Specifically, assay plates are made by coating the wells of a 96-well ELISA  
15   plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody  
20   detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

          A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants  
25   obtained in Example 31 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

          After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit)  
30   antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the

Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased  
5 fluorescent signal over background indicates a phosphorylation by polypeptide of the present invention or a molecule induced by polypeptide of the present invention.

*Example 42: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation*

10 This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond.  
15 Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in  
20 such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or  
25 agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells  
30 are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-

glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that  
5 can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10  $\mu$ l of prepared cytokines, 50  $\mu$ l of the supernatants prepared in Example 31 (supernatants at 1:2 dilution = 50  $\mu$ l) and 20  $\mu$ l of diluted cells are added to the media  
10 which is already present in the wells to allow for a final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat  
15 using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined  
20 via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene  
25 therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell  
30 proliferation and/or to decrease the inhibition of cell proliferation in the presence of

cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and

5 "Infectious Disease" sections above, and elsewhere herein.

*Example 43: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)*

10 The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  integrin

15 receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

25 Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of  $0.2 \mu\text{g}/\text{cm}^2$ . Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem

cells is to be expected. Gene products of the invention (e.g., including, but not limited to, polynucleotides and polypeptides of the present invention, and supernatants produced in Example 31), are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment ( 5% CO<sub>2</sub>, 7% O<sub>2</sub>, and 88% N<sub>2</sub> ) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular polypeptide of the present invention is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene encoding said polypeptide may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of

interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

*Example 44: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation*

10 The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or  
15 smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without  
20 co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5  $\mu$ g/ml  
25 hEGF, 5mg/ml insulin, 1 $\mu$ g/ml hFGF, 50mg/ml gentamycin, 50  $\mu$ g/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours, culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50 $\mu$ g/ml Amphotericin B,  
30 0.4% FBS. Incubate at 37°C until day 2.



On day 2, serial dilutions and templates of the polypeptide of interest are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or polypeptides  
5 of the present invention and incubate at 37°C/5% CO<sub>2</sub> until day 5.

Transfer 60µl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4°C until Day 6 (for IL6 ELISA). To the remaining 100 µl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the  
10 culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON  
15 at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of  
20 diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker. Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT.  
25 Plates are again washed with wash buffer and blotted on paper towels. Add 100 µl/well of Enhancement Solution and shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay are tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the  
30 polypeptide of the present invention may be involved in dermal fibroblast

proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the polynucleotide/polypeptide of the present invention which gives a positive result. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the present invention and polynucleotides of the present invention may be used in wound healing and dermal regeneration, as well as the promotion of vasculargenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides and polynucleotides of the invention may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides and polynucleotides of the invention may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

*Example 45: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells*

5           The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1  
10 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor  
15 participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the  
20 plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells.  
25 Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20  $\mu$ l of diluted ExtrAvidin-Alkaline  
30 Phosphotase (1:5,000 dilution, referred to herein as the working dilution) are added to

each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

15 *Example 46: Alamar Blue Endothelial Cells Proliferation Assay*

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with

GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM ) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days.

5 After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and

10 changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and

15 inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

20

*Example 47: Detection of Inhibition of a Mixed Lymphocyte Reaction*

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides).

25 Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and

natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM<sup>®</sup>, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50  $\mu$ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1  $\mu$ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10  $\mu$ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1  $\mu$ C of [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

5       The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both  
10 incorporated herein by reference in their entireties. Moreover, the hard copy of and the corresponding computer readable form of the Sequence Listing of Serial No. 60/124,270 are also incorporated herein by reference in their entireties.

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Applicant's or agent's file reference number	PA103PCT	International application no.
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209059</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
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**ATCC Deposit No. 209059****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 209059

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

414

Applicant's or agent's file reference number	PA103PCT	International application
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B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>20 May 1997</b>	Accession Number <b>209060</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
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**ATCC Deposit No. 209060****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner. the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 209060

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application N
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209061</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

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**ATCC Deposit No. 209061****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**Page 2**  
**ATCC Deposit No. 209061**

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

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#### **NETHERLANDS**

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Applicant's or agent's file reference number	420 PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>20 May 1997</b>	Accession Number <b>209062</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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**ATCC Deposit No. 209062****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 209062

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application i
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209063</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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**ATCC Deposit No. 209063****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**Page 2**

**ATCC Deposit No. 209063**

### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

426

Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209064</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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<b>RO/US 09 MAR 2000</b>
Authorized officer <b>Yolanda Harrod</b> <b>PCT/Internat'l Appl Processing Div.</b> <b>(703) 305-9670</b>

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**ATCC Deposit No. 209064****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



Page 2

ATCC Deposit No. 209064

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

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#### **NETHERLANDS**

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209065</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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Authorized officer <u>Jolanda Harroo</u> <u>PCT/Internat'l Appl Processing Div.</u> <u>(703) 305-3070</u>

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**ATCC Deposit No. 209065****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

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**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 209065

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

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The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>20 May 1997</b>	Accession Number <b>209066</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application <b>RO/US 00 MAR 2000</b> Authorized officer <b>Yolande Harrod</b> <b>PCT/Int'l Appl Processing Div.</b> <b>(202) 305-3870</b>	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer
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**ATCC Deposit No. 209066****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**Page 2****ATCC Deposit No. 209066****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <p style="text-align: center;">American Type Culture Collection</p>	
Address of depositary institution (including postal code and country) <p style="text-align: center;">10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</p>	
Date of deposit <p style="text-align: center;">20 May 1997</p>	Accession Number <p style="text-align: center;">209067</p>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application <b>RO/US 03 MAR 2000</b> Authorized officer <b>Yolanda Harrod</b> <b>PCT/Internat'l Appl Processing Div.</b> <b>(202) 305-3670</b>	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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**ATCC Deposit No. 209067****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**Page 2****ATCC Deposit No. 209067****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>20 May 1997</b>	Accession Number <b>209068</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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**ATCC Deposit No. 209068****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 209068

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>20 May 1997</b>	Accession Number <b>209069</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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**ATCC Deposit No. 209069****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 209069

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>12 January 1998</b>	Accession Number <b>209579</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")     	

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<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <b>30 MAR 2000</b> <b>159</b>	Authorized officer

**ATCC Deposit No. 209579****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2  
ATCC Deposit No. 209579

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution  American Type Culture Collection	
Address of depositary institution (including postal code and country)  10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit  12 January 1998	Accession Number  209578
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
RO/US 03 MAR 2000	
Authorized officer Yolanda Harrod PCT/Internat'l Appl Processing Div. 7030 305-3070	Authorized officer

**ATCC Deposit No. 209578****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2  
ATCC Deposit No. 209578

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

450

Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <p style="text-align: center;">American Type Culture Collection</p>	
Address of depositary institution (including postal code and country) <p style="text-align: center;">10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</p>	
Date of deposit <p style="text-align: center;">16 July 1998</p>	Accession Number <p style="text-align: center;">203067</p>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div>For receiving Office use only</div> <div><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div><b>RO/US 03 MAR 2000</b></div> <div>Authorized officer: <b>Harrold</b> <b>PCT/Int'l Appl Processing Div.</b> <b>1203 305-3670</b></div>	<div>For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer:</div>
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**ATCC Deposit No. 203067****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



**Page 2**  
**ATCC Deposit No. 203067**

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>16 July 1998</b>	Accession Number <b>203068</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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**ATCC Deposit No. 203068****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2

ATCC Deposit No. 203068

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

456

Applicant's or agent's file reference number	PA103PCT	International application?
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <p style="text-align: center;">American Type Culture Collection</p>	
Address of depositary institution (including postal code and country) <p style="text-align: center;">10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</p>	
Date of deposit <p style="text-align: center;">1 February 1999</p>	Accession Number <p style="text-align: center;">203609</p>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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<input checked="" type="checkbox"/> This sheet was received with the international application <b>RO/US 05 MAR 2000</b>	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <b>Yolanda Harrod</b> PCT/Internat'l Appl Processing Div. (703) 305-667	Authorized officer

**ATCC Deposit No. 203609****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2  
ATCC Deposit No. 203609

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

459

Applicant's or agent's file reference number	PA103PCT	International application no.
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>1 February 1999</b>	Accession Number <b>203610</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application <b>RO/US J 6 MAR 2000</b> Authorized officer <b>Yolanda Harrod</b> <b>PCT/Internat'l Appl Processing Div.</b> <b>(703) 305-3670</b>	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer
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**ATCC Deposit No. 203610****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2  
ATCC Deposit No. 203610

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

462

Applicant's or agent's file reference number	PA103PCT	International application?
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <p style="text-align: center;">American Type Culture Collection</p>	
Address of depositary institution (including postal code and country) <p style="text-align: center;">10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</p>	
Date of deposit <p style="text-align: center;">17 November 1998</p>	Accession Number <p style="text-align: center;">203485</p>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only	<input type="checkbox"/> For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
<p style="text-align: center;"><b>RO/US 08 MAR 2000</b></p>	
Authorized officer <p style="text-align: center;">Yolande Harrod PCT/Internet Appl Processing Unit (703) 305-3670</p>	Authorized officer

**ATCC Deposit No. 203485****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

Page 2  
ATCC Deposit No. 203485

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

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#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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Applicant's or agent's file reference number	PA103PCT	International application f
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>18 June 1999</b>	Accession Number <b>PTA-252</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application <b>RO/US 03 MAR 2000</b> Authorized officer <b>Yolanda Harrod</b> <b>PCT/Internat'l Appl Processing Div.</b>	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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**ATCC Deposit No. PTA-252****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

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ATCC Deposit No. PTA-252

#### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### **SWEDEN**

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#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



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Applicant's or agent's file reference number	PA103PCT	International application No.
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>10801 University Boulevard Manassas, Virginia 20110-2209 United States of America</b>	
Date of deposit <b>18 June 1999</b>	Accession Number <b>PTA-253</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application <b>RO/US 05 MAR 2000</b> Authorized officer <b>Nolanda Harrod</b> <b>PCT/Internat'l Appl Processing Div.</b> <b>(703) 305-3670</b>	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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**ATCC Deposit No. PTA-253****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

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ATCC Deposit No. PTA-253

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

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#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	471 PA103PCT	International application #
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>72</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution  American Type Culture Collection	
Address of depositary institution (including postal code and country)  10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit  22 December 1999	Accession Number  PTA-1081
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application <b>RO/US 03 MAR 2000</b> Authorized officer <b>Yolanda Harrod</b> <b>PCT/Internat'l Appl Processing Div.</b> <b>(703) 305-3670</b>	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on:  Authorized officer
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**ATCC Deposit No. PTA-1081****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**FINLAND**

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**UNITED KINGDOM**

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Page 2

ATCC Deposit No. PTA-1081

#### **DENMARK**

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*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
- 5
- (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
  - 10 (c) a polynucleotide encoding a polypeptide fragment of a polypeptide encoded by SEQ ID NO:X or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
  - 15 (d) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
  - (e) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
  - 20 (f) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X, having biological activity;
  - (g) a polynucleotide which is a variant of SEQ ID NO:X;
  - 25 (h) a polynucleotide which is an allelic variant of SEQ ID NO:X;
  - (i) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
  - (j) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide
  - 30

sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.



10. The recombinant host cell of claim 9 comprising vector sequences.
11. An isolated polypeptide comprising an amino acid sequence at least  
5 95% identical to a sequence selected from the group consisting of:
- (a) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
  - (b) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone, having biological activity;
  - 10 (c) a polypeptide domain of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
  - (d) a polypeptide epitope of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
  - (e) a full length protein of SEQ ID NO:Y or of the sequence encoded by the  
15 cDNA included in the related cDNA clone;
  - (f) a variant of SEQ ID NO:Y;
  - (g) an allelic variant of SEQ ID NO:Y; or
  - (h) a species homologue of the SEQ ID NO:Y.
- 20 12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.
13. An isolated antibody that binds specifically to the isolated polypeptide  
25 of claim 11.
14. A recombinant host cell that expresses the isolated polypeptide of claim 11.
- 30 15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

5           16.     The polypeptide produced by claim 15.

17.     A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

10

18.     A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

15

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19.     A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

20

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

25

20.     A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

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21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.
22. A method of identifying an activity in a biological assay, wherein the method comprises:
- 5 (a) expressing SEQ ID NO:X in a cell;
- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.
- 10 23. The product produced by the method of claim 20.